THE UNIVERSITY *of* EDINBURGH

The use of genetic variation in short-term feeding behaviour in broiler breeding programmes

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Declaration

I declare that this thesis is all my own work, except where acknowledged. Neither this work, nor any part of it, has previously been submitted for any other degree or professional qualification.

J. A. Howie 22nd February 2010

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Thesis Abstract

Genetic variation between individuals is of great importance for the development of breeding programmes, to select for animals with the most favourable traits. Many production companies routinely measure the feed intake of their animals, in order to calculate efficiency traits such as feed conversion ratio. The development of electronic feeders which automatically record individual intake on a visit-by-visit basis now allows the short-term feeding behaviour of animals to be monitored and analysed as another source of variation between individuals. Due to differences in the resolutions of these feeders as a measurement tool, a standard unit of feeding event needs to be estimated to allow for comparisons between studies. Different models for estimation of the defining value of a meal, the meal criterion, have been used, with the most recent incorporating the change in satiety with time since last feeding as part of the model.

In this study I developed a new methodology, based on these models, for use when a within-meal population of intervals cannot be easily modelled. I then used this model for application to data from four lines of broiler chickens to estimate meal criteria and compare feeding behaviour within and between the lines. Significant differences were found between fast and slow growing birds, with the faster growing birds having fewer but larger meals than the slower growing birds. However, the lines showed similar structure and bouting of their feeding behaviour, indicating that the fundamental controls of feeding behaviour, such as hunger and satiety, in these lines had been unaltered despite intensive selection for growth. The models were also applied across poultry species, kept in different experimental conditions. A similar

structure to the feeding behaviour was found across all these species, with all showing clear separation of feeding events into bouts.

In order to estimate the potential use of these behavioural observations in a breeding programme, the heritabilities and genetic correlations with existing performance traits were calculated for the four broiler lines. Heritabilities of all feeding behaviour traits were found to be moderate to high, and very similar across the lines. Correlations with performance traits, however, were low, meaning that there were no clear links of the traits with the current production goals investigated. This indicates that past selection for production has had limited impact on feeding behaviour and also that potential selection for feeding behaviour will have little effect on production gains.

To identify the areas of the genome controlling feeding behaviour, traits were associated with a SNP panel. Many regions were found to have highly significant association with feeding behaviour traits, with the most highly correlated traits showing associations with the same regions, suggesting pleiotropic effects of genes in these regions. Future work in this area should include identification of individual genes controlling feeding behaviour to allow prediction of the effects of selection for favourable feeding behaviour on other traits, and comparison of the genotypes of different lines of broilers, to further understand the control of feeding behaviour.

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CHAPTER 1

General Introduction

1.1 Why analyse feeding behaviour?

The provision of feed constitutes a major cost in livestock production. This is most evident in the poultry industry, where feed accounts for around 60% to 70% of the total costs of production (Waller 2007). One of the main foci of current breeding goals is to improve efficiency of feed utilisation and thus reduce the financial costs associated with intensive rearing of birds (McKay 2007). Individual daily feed intake is, therefore, frequently measured by breeding companies because it provides essential information for the calculation of feed efficiency.

Variation in daily feed intake is directly related to variation in the structure of short-term feeding behaviour (STFB), i.e. the number and distribution of feeding bouts during a day and the average intake per bout (Meguid et al., 1998). Analysis of this behaviour can serve various purposes, such as the testing of hypotheses on the control of feed intake (e.g. Tolkamp et al., 2002), or the early identification of health and welfare problems (e.g. Gonzalez et al., 2008). In addition, variation in STFB between individuals can be quantified with the potential to identify traits that can be used for genetic improvement of livestock via breeding programmes.

1.2 How is STFB measured?

1.2.1 Recording of STFB

STFB is measured in terms of feeding events, the definitions of which vary between studies, mainly depending on the equipment used for recording this behaviour. Among the techniques used in previous studies are (Tolkamp and Kyriazakis 1999): direct visual observation (e.g. horses - Mayes and Duncan 1986), video film analysis (e.g. goats - Adenuga et al., 1991), jaw movement recordings (e.g. cattle - Metz 1975, Dado and Allen 1993), measurement of food

container weights at regular intervals (Dawson and Mayne 1998) and by using specially designed computerised feeders that record visits, allowing recognition of each animal as it enters (e.g. Morgan et al., 2000; Tolkamp et al., 2000). The technique used has a large effect on the number of feeding events recorded, and thus the intake per event. This makes robust comparisons between studies that use different recording methods and/or resolutions very difficult (Tolkamp et al., 2000).

Breeding companies often use computerised feeders in combination with electronically tagged animals to estimate individual feed intake under group-housing conditions. The basic feeding event in such recording systems is a visit by an animal to a feeder, and numerous studies analyse STFB exclusively in these terms (e.g. Barbato et al., 1980, Baumung et al., 2006). It has been shown, however, that relatively small changes in experimental conditions (e.g. number of animals per feeder or feeder construction) can have very large effects on STFB that is analysed in terms of visits, without any effect on daily feed intake (e.g. Nielsen et al., 1995). It is therefore questionable whether a visit to a feeder in the best unit in which STFB can be analysed.

It is generally thought that animals organise their feeding behaviour into feeding bouts, or meals (Tolkamp et al., 1998). When measured with electronic feeders, a feeding bout or meal will consist of one or more visits to feeders and, in the latter case, of one or more short non-feeding intervals within a meal. Meals are thought to be separated by longer non-feeding intervals. Tolkamp and colleagues (2000) showed that a meal was a more biologically relevant unit for analysis of feeding behaviour than visits to feeders, at least when a meal criterion can be identified to correctly group visits into feeding bouts.

1.2.2 Methods for defining a meal

The definition of a meal is not immediately obvious, and a variety of methods have been proposed over the years for estimation of a "meal criterion". Such a meal criterion is an estimate of the longest interval that can be considered as part of a meal. All longer intervals are then intervals between meals. Some studies have used purely arbitrary values for a meal criterion (Kissileff 1970, Bokkers and Koene 2003), whereas many others use a variety of mathematical techniques to define what constitutes a meal (e.g. Simpson and Ludlow 1985, Langton et al., 1995). As the definition of a meal can profoundly affect the results of STFB analysis (Demaria-Pesce and Nicolaidis 1998; Zorrilla et al., 2005), it is important to demonstrate that a biologically relevant meal criterion has been estimated.

Until recently, the most popular methods for meal criterion estimation were log-survivorship (e.g. Slater and Lester 1982) and log-frequency analysis (e.g. Langton et al., 1995). Log-survivorship analysis fits two negative exponential distributions to the cumulative frequencies of intervals between feeding events, and the meal criterion is estimated at the point where the two distributions cross. Because of the usually very large numbers of very short intervals, the original frequencies, as well as the fitted model, are subsequently log-transformed to obtain a more informative graphical picture. Figure 1, taken from Slater and Lester (1982) illustrates this method. Log-frequency analysis works in a very similar manner, except that the double negative exponential model is fitted to frequencies of interval length instead of cumulative frequencies.

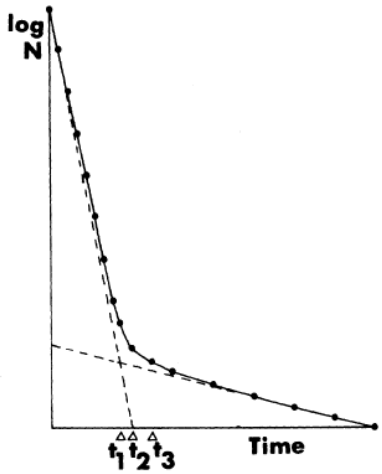

Figure 1: Log-survivorship curve, taken from Slater and Lester (1982). Meal criteria are generally estimated at the point where the frequency distributions cross, minimising the number of intervals mis-assigned to either within or between meals.

Both these models are based on the underlying assumption that the probability of an animal initiating feeding does not change with time since the last feeding event, which is contradictory to the concept of the effects of satiety on feeding behaviour. Although the term "satiety" does not have a very precise physiological meaning (Forbes 1995), it is generally used to indicate a state in which the animal is not stimulated to eat. It was defined by LeMagnen in 1985 as "..a passive state of no hunger". The concept of satiety and hunger as feed intake controllers is that animals feed until they are satiated. Thus satiety mechanisms are thought to control the point at which an animal stops eating. Feed consumption is, however, thought to be initiated by levels of hunger that increase with time since the last feeding occurred. Under these assumptions, the probability of an animal starting to feed is expected to increase with time since the last meal, as observed in experiments in cows (Tolkamp et al., 2000). This contrasts strongly with the underlying assumptions of log-survivorship and log-frequency analysis.

To incorporate the assumptions of the satiety concept into a model for determining a meal criterion, Tolkamp and colleagues (1998) originally proposed the use of a double log-normal model. In this model, the two log-normals represent the distributions of log-transformed within- and between-meal interval lengths respectively, and a meal criterion is estimated at the point where these two distributions cross (Figure 2).

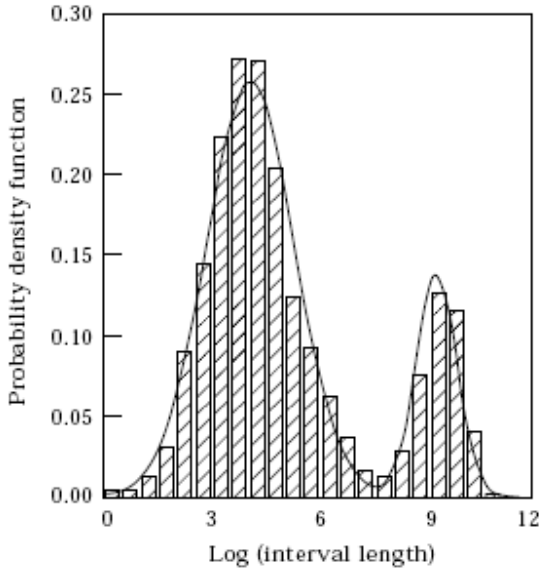

Figure 2: Probability density function of the double log-normal model from Tolkamp et al (1998). The graph shows the observed distribution of $\log_e$ transformed intervals (measured in seconds) between visits to feeders divided by bin width (i.e. 0.5 $\log_e$ units), and the fitted model (black line).

Further experiments by Tolkamp & Kyriazakis (1999) led to the incorporation of drinking behaviour into meal pattern analysis, as many animals often drink in association with meals (e.g. rats take in 70-85% of their daily water intake during a meal - Kissileff 1970). When data were used from cows that drank during meals, the inclusion of a third log-normal distribution into the model gave a significantly improved fit (Tolkamp and Kyriazakis 1999). As the water

troughs were situated away from the feeders, intervals within a meal during which drinking occurred were relatively longer than those in which no drinking occurred. The meal criterion was calculated using the model parameters to estimate the point at which the distributions representing within-meal drinking intervals and between-meal intervals crossed.

One major problem with this three-log-normal model was that after back-transformation to a real time-scale, the model predicted a meal starting probability that first increased and then decreased with time since the last meal. This is still in conflict with the expectations based on the satiety concept. Yeates and colleagues (2001) therefore proposed a log-Weibull distribution for modelling the between meal intervals, because the parameters of this function accurately predicted the expected as well as observed increase in starting probability with time since feeding last (Figure 3).

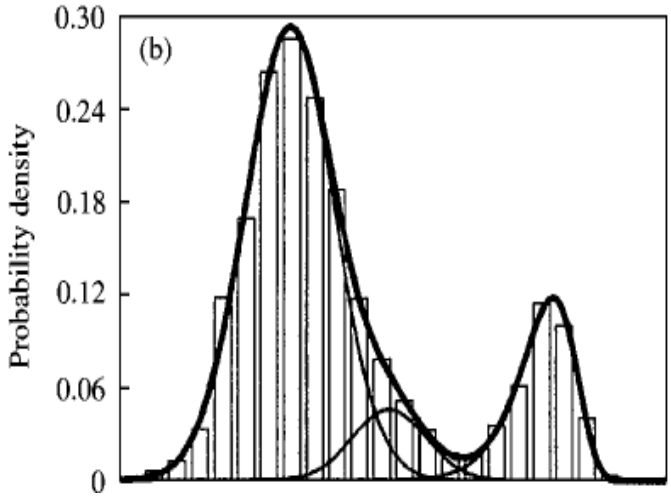

Figure 3: The double log-normal and log-Weibull distribution model from Yeates et al (2001). As in Figure 2, this graph shows the probability density of the log-transformed intervals between visits (bars), the three fitted distributions (thin lines) and the overall model (thick line).

These log-normal and log-Weibull models require, however, that the underlying populations of interval lengths are readily identifiable, which may not always be the case (see below). Therefore the first aim of the current study was to adapt these methods for such cases, which is outlined in Chapter 2.

1.3 Variation in STFB in broiler chickens

1.3.1 Current progress in the broiler industry

Intensive selection in the broiler industry over the past 40 years has led to large physical changes in the birds. Male broiler chickens currently grow from 40 grams to 2.6 kilograms in just 42 days, with an annual improvement in live weight gain of around of 60 grams over this time period (McKay et al., 2000). Associated with these increased growth rates are changes in body conformation, especially increased breast meat yield and carcass weights. With this rapid growth have come some detrimental health effects, such as increases in leg defects (Weekes et al., 1997) and immunological problems (Rauw et al., 1998). Current breeding programmes select, however, for robust birds to minimise adverse effects of rapid growth and to provide fast-growing but healthy birds.

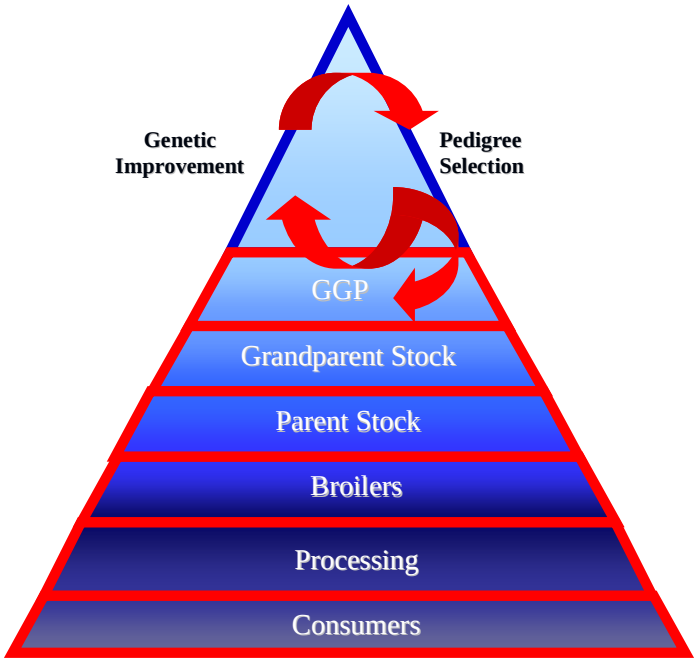

Figure 4: Hierarchical structure of the broiler industry. At the top of the pyramid are the developmental lines, where selection and genetic improvement takes place. Birds then enter either into the production part of the pyramid as great-grandparent (GGP) stock or are bred to maintain the developmental lines.

1.3.2 Selection in the broiler industry

The data for this study were provided by Aviagen Ltd from trials conducted on their developmental lines. These lines are maintained as distinct pure strains which are crossbred to produce hybrid birds for production purposes, shown in Figure 4. Selection of birds in each line is carried out based on the individual bird's performance, as well as from information obtained from the pedigree and the results of sibling trials carried out in different environmental conditions. Birds are then selected for improvement of the line performance, as well as being crossbred with other lines to produce great-grandparent stock for entry into the food production chain. Selection is carried out in the pure bred lines as each line has differing

performance goals. For example, the main goal of a “male” line may be increased body weight gain, whereas the focus will be more on increased fertility and egg production for a “female” line. Selection decisions implemented in the developmental lines typically take four years to reach the consumer level of the pyramid.

As feed is the major cost in poultry production, decreasing feed costs whilst maintaining or improving body weight gain is one of the main current production targets (McKay 2007). If a bird is using feed more efficiently, it will need to consume less feed per kilo of body weight gained. This is measured commercially as the feed conversion ratio (FCR), which is the weight of feed in kilograms that is required to gain one kilogram of body weight. Thus a lower ratio means that less feed is required for body weight gain. FCR was chosen as the measure of feed efficiency, rather than either its inverse, feed conversion efficiency (FCE), or residual feed intake (RFI), as it is the unit most commonly used commercially and therefore the most relevant to industry. Measurement of FCR in commercial situations is difficult, as animals are normally group-housed, which makes individual feed consumption recording impossible without some sort of electronic identification system of the animals in the group. A variety of systems for automated recording of visits to feeders by group-housed but electronically tagged individuals have been successfully developed (e.g. DeHaer et al., 1992, Musial et al., 1999, Green et al., 2003). In poultry systems, birds are fitted with a transponder on the wing or leg which can be detected and read by an antenna in the feed station when a bird enters and leaves a feeder. Feed consumption is also measured electronically using scales linked up to a computer, thus intake per bird can be calculated. Figure 5 shows a photograph of the feed stations used by Aviagen for this trial.

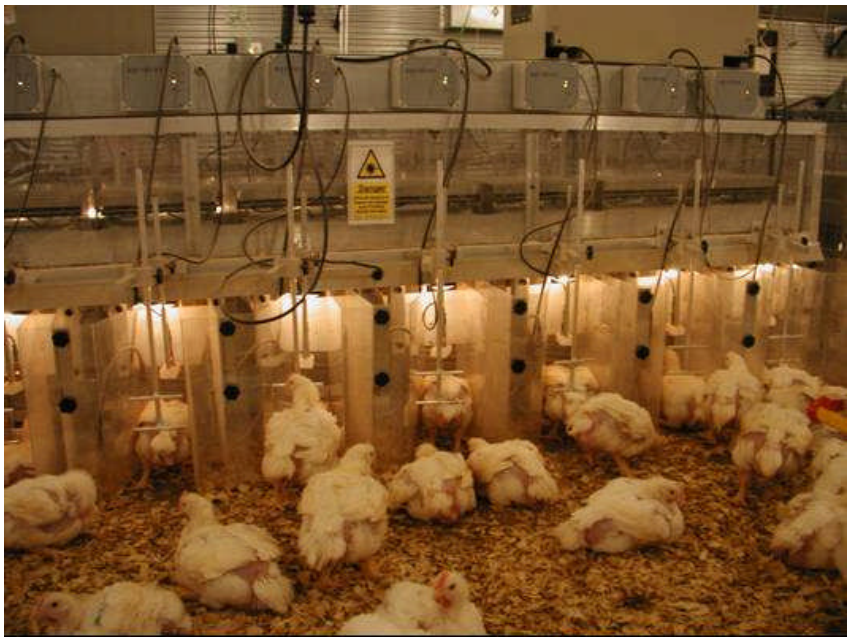

Figure 5: A photograph showing broilers using the feeders in a feed station.

1.3.2.1 Phenotypic variation in developmental lines

Birds from each of the developmental lines spend three weeks in pens equipped with electronic feeders, which automatically record visits to feeders of individually identifiable birds. Although these feeders were primarily developed to measure average daily feed intake, they provide a unique opportunity to evaluate the differences in feeding behaviour. Differences can be measured both between lines, and between individuals within each line, to determine whether there is enough variation in feeding behaviour for potential use in a selection programme. Not all birds will exhibit the same feeding behaviour, as they are housed in a group situation and dominance hierarchies will play a role (Banks et al., 1979). Animals also have individual preferences, so that the same daily intake can be achieved by either many small meals or by fewer but larger meals (Meguid et al., 1998). The relevance of this behaviour as a potential selection criterion depends on whether there is a link of a certain type of behaviour

with current production goals, i.e. which is the most favourable behaviour. It also gives the opportunity to investigate whether past selection to produce the different genetic lines has led to differences between lines in feeding behaviour. Some previous studies on comparisons between strains in other species have shown that the lines with larger body size tend to have fewer meals (e.g. Labroue et al., 1999). Other studies, frequently using other definitions of feeding behaviour units, have come to different conclusions (e.g. Barbato et al., 1980, Petersen and McCarthy 1981). This highlights the need for a standardised unit of measurement for feeding behaviour analysis, as biologically relevant comparisons between studies are not possible, as any differences found may be due to differences in measuring technique.

1.3.2.2 Genetic variation in traits

Current selection programmes are not based on phenotypic data alone, but use estimated genotypic breeding values (EBVs). These EBVs take into account the phenotype of the individual animal, as well as information from the pedigree. As genotyping is an expensive process, not every animal can be genotyped, and information from a pedigree is thus crucial. In order to evaluate the potential of a trait for use in a breeding programme, estimates are made of the genetic variance as well as the heritability of the trait and of covariances with other traits. Heritability is defined in this thesis as "the extent to which phenotypes are determined by the genes transmitted from the parents" (Falconer and Mackay 1996). It is calculated as the ratio of the additive genetic variance (V_A) and the phenotypic variance (V_P) of the trait. The genetic variance is defined as the amount of variance in a trait which is due to differences in the genetic makeup of the individuals, and the phenotypic variance is the sum of the genetic variance and the variance due to differences in environmental conditions (environmental variance).

The estimation of breeding values of individuals requires complex statistical mathematics, and current selection programmes often use best linear unbiased prediction (BLUP) as a prediction mechanism. This method simultaneously estimates both the fixed effects and the breeding values, and can also estimate the genetic variances of the traits using restricted maximum likelihood (REML) techniques (Falconer and Mackay 1996; Simm 1998). If an individual animal model (a specialised form of BLUP) is used, a breeding value can be predicted for every animal with measurements for a trait, and relationships between all the animals are taken into account. BLUP works by using matrices of variances and covariances of traits to find best estimates for breeding values and heritabilities. Thus the reliability of any BLUP output is dependent on the amount and accuracy of the data that was used for the analysis.

For this study, large data sets were available for different broiler lines that allowed quantification of genetic variances, co-variances and heritabilities of STFB traits (Chapter 5). No previous studies have investigated the genetics of STFB in chickens, but there have been a few such studies in pigs and cattle (e.g. Hall et al., 1999, Robinson and Oddy 2004). The results of these studies are quite varied, with heritability estimates ranging from 0.05 to 0.5 for most feeding behaviour traits. These studies frequently differed in their method of recording STFB, as well as their definitions for the traits. It is therefore very difficult to draw general conclusions from a collection of such studies. Similarly, considerable differences in estimates of genetic correlations with performance traits such as FCR and body weight have been reported, which are again difficult to interpret for the same reasons. Since animals generally structure their feeding behaviour to occur clustered into bouts (usually referred to as meals), methodologies that were developed during this study to correctly identify meals in one genetic line of broilers are discussed in Chapter 2. These methodologies are subsequently applied to

different broiler lines in Chapter 3 to test hypothesis about the possible effects of selection for performance traits on STFB. In Chapter 4, the suitability of methods developed with data obtained from broilers for use in two other species, turkeys and ducks, is assessed. In Chapter 5, the heritabilities of feeding behaviour traits, and their genetic correlations with each other and two major performance traits (body weight and total feed intake) are estimated.

1.4 The use of single nucleotide polymorphisms in livestock breeding

With the completion of the chicken genome mapping project in 2004 (International Chicken Polymorphism Map Consortium), it is now possible to link specific areas of the genome to traits of interest. Over 2.8 million single nucleotide polymorphisms were identified in the chicken genome, which allows large genome-wide association studies of traits with these identifiable regions to be carried out. A single nucleotide polymorphism (SNP) is a variation in one nucleotide of the DNA sequence between individuals of the same species. These variations can occur in any part of the genome, but if they occur within a gene they may alter the amino acid produced and thus have an effect on the resulting protein. Most SNPs are bi-allelic, meaning that in a population there are two different nucleotide bases at the same position but there may be more than two different bases found at the same position in some SNPs, which are then termed multi-allelic (Falconer and MacKay 1996).

SNPs are reference points in the genome that allow comparisons between individuals to better understand the genetic control of traits. In order to compare individuals, they need to be genotyped using SNP chips to identify the alleles present at each known SNP locus. Genotyping of individuals, however, is an expensive process, so it is used in conjunction with a known pedigree to identify the best animals to breed from. SNPs are useful in breeding

programmes for traits which either have a low heritability or are difficult to measure in animals intended for breeding purposes (e.g. carcass composition, which can only be measured reliably after death – Dekkers 2004). In these cases, marker assisted selection can be used to select for the trait of interest, if linked to known SNPs. This requires that the potential breeding individuals are genotyped for presence or absence of the alleles linked to the trait of interest.

1.4.1. Methods for association of SNPs with phenotypic traits

SNP association studies can be used to scan the genome for quantitative trait loci (QTLs) which are significantly associated with traits of interest. One method to investigate these associations is through linkage disequilibrium (LD). Linkage disequilibrium occurs when there are non-random associations between polymorphisms at different loci. If known SNP locations are associated with traits, this indicates that genes controlling the trait are located in a similar area of the genome to the SNP. Therefore SNP association studies can lead to increased understanding of the genetic control of traits, and the effects that selection for other traits will have. The final experimental chapter, Chapter 6, looks at association of SNPs with feeding behaviour and performance traits in an attempt to identify regions of the genome which are controlling short-term feeding behaviour and to further understand the effects that selection for body weight and feed intake is having on feeding behaviour.

1.5 Thesis aims

The main aims of this thesis were:

- To determine the structure of short-term feeding behaviour in broilers
- To determine whether current models of short-term feeding behaviour are applicable to broilers
- To compare short-term feeding behaviour in lines of broilers which differ in their selection goals
- To compare short-term feeding behaviour of broilers with that of other poultry species, and to determine whether models for estimation of meal criteria are applicable to other poultry species
- To estimate the heritabilities and genetic correlations of short-term feeding behaviour traits, and their associations with current selection goals
- To find regions of the genome associated with short-term feeding behaviour traits using SNP association studies.

1.6 Thesis outline

This thesis is divided into 6 further chapters. The second chapter describes and discusses novel methodologies that were developed for estimating meal criteria in broiler chickens, and tests these methodologies against a data set obtained from dairy cows. Chapter 3 looks at the differences in feeding behaviour at the phenotypic level between four lines of broilers, resulting from differences degrees of selection for growth. These data are then used to test hypotheses on the level of hunger experienced by these different lines of birds. The fourth chapter extends this study to compare feeding behaviour of broilers with two other poultry species (turkeys and ducks) and introduces a further methodological development based on disaggregation of previously pooled data sets. Heritabilities of feeding behaviour traits and their genetic correlations with performance traits are quantified in Chapter 5, and associations of the feeding behaviour traits with SNPs are described in Chapter 6. Finally, a general

discussion in Chapter 7 combines the findings of each of the experimental chapters into overall conclusions and outlines potential future research that has been suggested by this study. There is no separate literature review, as chapters are written in the style of scientific papers and each contains a review and associated bibliography of the relevant literature for that chapter.

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CHAPTER 2

A novel flexible method to split feeding behaviour into bouts

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Chapter 2 – Splitting feeding behaviour into bouts

2.1 Abstract

Before meal patterns can be analysed properly, a biologically relevant meal-criterion must be determined in order to group short-term feeding behaviour into meals. Existing methodologies are based on modelling of the frequency distributions of intervals between feeding events but these methods cannot be used if the proper distributions cannot be clearly identified. For such cases I developed two new methods – 1: based on the analysis of the distribution of between-meal interval lengths only and 2: based on the analysis of changes in the probability of animals starting to feed with time since the last feeding event. Both methods were developed using a data set of over 700,000 records of visits to feeders obtained with broilers (*Gallus gallus*) aged between 2 and 5 weeks. The two methods resulted in meal criteria estimates of 20.1 and 17.5 min respectively, which, when applied to the data set, gave statistically significant but very small differences in meal characteristics. The new methods were tested against an independent cow (*Bos taurus*) data set and the resultant meal criteria compared with those predicted by an existing method. The two novel methods estimated meal criteria for cows at 27.9 and 35.5 min, compared with 28.9 min for the existing method. Again, these differences in meal criteria resulted in only very small differences in meal characteristics. Even though meal criteria were relatively similar for birds and cows, characteristics such as average daily number of meals (10.9 and 5.9), meal size (12.5 g and 7.4 kg) and meal duration (7.7 and 31.4 min) were very different. The analyses show that, if the appropriate distributions of intervals cannot be identified, meal criteria can still be estimated for species as diverse as mature cows and young birds by the novel methodologies developed here.

2.2 Introduction

Studies of short-term feeding behaviour (STFB) are concerned with the feed intake patterns of animals at the level of feeding events and meals. Analysis of STFB can have various objectives. Because daily intake is determined by meal frequency and amount of feed consumed per meal (Meguid et al., 1998), STFB can be used to test hypotheses on the control of feed intake and diet selection, including the roles of hunger and satiety (e.g. LeMagnen and Tallon, 1963; Tolkamp et al., 2002; Bokkers and Koene 2003) . In addition, analysis of STFB can assist in identifying relevant traits for incorporation into selection programmes (e.g. Rauw et al., 2006) and aid in identification of health and welfare problems (Sowell et al., 1998; Gonzalez et al., 2008).

Many different techniques have been used for recording STFB, ranging from direct visual observations (e.g. Slater 1974; Simpson and Ludlow 1986; Mayes and Duncan 1986) and records of jaw movements (e.g. Metz 1975), to the use of specially designed computerised feeders that allow the recording of feeding behaviour of individual animals, even within large groups (e.g. De Haer and Merks 1992; Nielsen et al., 1996; Bley and Bessei 2008). The technique used to measure STFB, and the resolution of this technique, both have a large effect on the definition, the biological significance and the number and average length of the shorter feeding events that are recorded (Tolkamp et al., 2000). Thus it is very difficult to compare results obtained in terms of feeding events from studies using different methods, as robust comparisons can only be made between results of studies with similar recording method and resolution (Demaria-Pesce and Nicolaidis 1998). Such problems can be avoided if meals are used as the basic unit of daily feed intake instead of short feeding events (Tolkamp et al., 2000). Because satiety is high at the end of a meal, the probability of animals starting a meal

immediately after terminating one will be low but is expected to increase with time (Yeates et al., 2001; Zorrilla et al., 2005). This pattern has not been found when using visits as the units of analysis. If an appropriate definition of a meal is used, meal pattern analysis should not be affected by the units in which feed intake was measured originally. While information from analysis of feeding event data can be useful in itself (Tolkamp et al., 2000), the grouping of events into meals may yield findings on the underlying control of feed intake which would be missed if the data were only analysed at event level.

In order to group data from visits to a feeder into meals, a meal criterion is needed which represents the shortest interval between visits that can be considered to be between separate meals (a between-meal interval). Interval lengths shorter than the meal criterion are therefore considered to be occurring within meals. Previously, meal criteria have either been defined in an arbitrary manner (e.g. Kissileff 1970; Bokkers and Koene 2003) or calculated from model parameters (e.g. Slater and Lester 1982; Bigelow and Houpt 1988; Tolkamp and Kyriazakis 1999a; Zorrilla et al., 2005). The method used to estimate meal criteria has a large effect on the values obtained and the use of these different meal criteria can subsequently have a considerable impact on the conclusions drawn from meal pattern analysis (Demaria-Pesce and Nicolaidis 1998; Zorrilla et al., 2005). Therefore it is important that a biologically relevant meal criterion is estimated in order to draw valid conclusions from meal pattern analyses.

Previous models did not always incorporate the expected effects of satiety on the structure of STFB (Tolkamp et al., 1998). Recent models that relied on normal and/or Weibull functions to describe the frequency distribution of log-transformed interval lengths are more consistent with the effects of satiety on STFB (Tolkamp et al., 1998; Tolkamp and Kyriazakis 1999b; Yeates

et al., 2001). These models, that can incorporate within-meal intervals during which animals drink (Tolkamp and Kyriazakis, 1999b; Yeates et al., 2001) require, however, that the functions to describe the distribution of intervals from both the within- and between-meal populations can be identified.

In this chapter I describe the development of new methodologies to estimate biologically appropriate meal criteria when suitable functions for the description of the distribution of intervals between visits cannot be identified. Firstly, I adapted the existing models of Yeates et al (2001), to make these suitable for use if only the appropriate function to describe the between-meal frequency distribution is known; this adaptation was tested against data obtained with female broiler birds. Using the same data set, I developed a novel methodology based on the changes in the probabilities of animals starting to feed with time since last feeding. This method can be applied for estimating meal criteria even if none of the functions describing between-feeding intervals can be identified. The general applicability of the novel methods that were developed on the basis of data obtained with birds was subsequently tested against an independent data set obtained with dairy cows, because it was the aim of this study to develop a general methodology that can be applied across species.

2.3 Materials and Methods

2.3.1 Data Set 1

2.3.1.1 Animals and housing

Records of visits to feeders (n = 701864) were obtained from 1058 female broiler birds from 14 to 35 days of age. The birds were all from three hatches of the same genetic line. The hatches were reared in three sheds, with the birds in shed one hatching a week earlier than those in shed two and two weeks earlier than those in shed three. Each shed contained three pens (3.0 m x 2.7 m) with two bell drinkers, eight feeders and a layer of wood shavings over the floor. This gave a density of approximately 14.5 birds per feeder, which was considered to be sufficient as the feeder occupancy throughout the trial was less than 70% during the light hours, thus giving the birds ample opportunity to feed. Previous tests to establish the optimum number of birds per feeder were performed by Aviagen and 14.5 was found to be the best density to allow maximum utilisation of the feeders without compromising access to feed.

Immediately after hatching, the birds were placed in pens with additional heating to allow them to adapt to the feeding system and environment. Although birds were able to feed from the electronic feeders from day 1, no data were recorded until day 14. During the rearing and experimental periods, temperature and lighting were maintained in line with commercial husbandry practice, with temperature gradually reduced from 32 ^{o}C to 19 ^{o}C and a 21 hour light period per day throughout the experimental period. This lighting period was chosen as this is the standard lighting hours used commercially for birds of this age and was in accordance with the Defra requirements for daily hours of darkness.Birds were handled for weighing at the start and the end of the experiment at 14 and 35 days of age, using a Sartorius weighing platform.

2.3.1.2 Feeders and feeding

An electronic feeding system based on similar principles as that described by Bley and Bessei (2008) was used. Each of the feeders (manufactured by Beck Plastics, Cumbernauld) consisted of a tray from which the birds could feed, and a back-bar to allow access of only one bird at a time. The width of the access to the feeder was altered in proportion to the size of the birds by changing side plates three times a week as the birds grew to allow comfortable entry of one bird into the feeder. Each of the feeders was automatically refilled so that there was approximately 20 g of feed available per feeder to the birds at any time. Between 1 and 11 days of age birds received a standard crumb diet and between 12 and 35 days of age, i.e. throughout the experimental period, they received a standard pellet feed.

Each bird was fitted with a small wing transponder bearing a unique identification code which was recorded as a bird entered and left a feed station using a radio frequency antenna system. A visit started when the antenna in the feeder detected and read the wing band transponder of the bird at the feeder. The visit ended when the bird left the feeder and therefore the wing band transponder was no longer in the range of the antenna. Scales were connected to each feeder to record the weight of feed in the tray at the beginning and end of each visit. Therefore the amount of feed consumed during the visit and the start and stop times of the visit could be assigned to an individual bird and this information was recorded by a computerised system and stored on a central server. Start and stop times were recorded to the nearest second, and weight consumed was recorded to the nearest 0.1 g. Also recorded for each visit were the date and the identification codes for the hatch, pen, feeder and bird. The interval lengths between subsequent visits by the same bird were calculated from these records. Previous observations at

the experimental facilities have suggested that the small wing transponder did not affect behaviour of the birds in any way.

2.3.1.3 Validation data from feed stations

In order to validate the data recorded by the feed stations, behavioural recordings and comparison weighings were made when testing the feed station design. Testing was carried out in 2003 over a 14 day period, by Aviagen staff. To confirm that the scales were recording the correct weights, feed from each feed place was weighed at the beginning and end of each day and this was compared with the amounts assigned to individual birds at each feed place over the course of a day to obtain a percentage accuracy for the feed station scales.

As the original data for confirming that the birds using the feed stations were being correctly identified by the antenna system, was not available data set 2 was used as an analysis to check that the bird IDs were being recorded accurately. These coloured birds could be identified by video recordings and thus could be used to check that the visit to the feeder recorded by the electronic system had been assigned to the correct bird.

2.3.1.4 Data screening

Any visits that were not assigned to specific birds, due to the wing band not being read correctly, were removed from the data set before analysis. This was less than 0.5% of the visits, during which less than 0.1% of feed consumption occurred for any of the hatches. Any values for weight of feed consumed less than -0.1 g (<0.5% of all visits for any hatch) were taken to be scale errors and excluded from meal size calculations.

2.3.2 Data Set 2

As drinking behaviour data was not recorded during collection of data set 1, a small experiment involving one pen of birds only was specifically designed for collection of data set 2 to test an assumption underpinning one of the model adaptations. The experimental set up was the same as during data collection for experiment 1 except that ten of the birds were randomly selected and colour-marked with a stripe across the back, using farm animal spray-paint (Ritchey super sprayline stock marker). The birds were re-sprayed weekly to allow easy identification. The spray was not observed to alter the behaviour of the animals in any way compared to the unmarked animals. Two colour CCTV cameras were connected to a time-lapse video recorder and set to record 24 hours per 3 hour video tape. Tapes were changed daily at around 8:30am and records were obtained for the entire three week experimental period. The time setting of the video recorders was synchronised with the time setting of the feeders. The time at which the coloured birds drank was recorded and associated with the relevant intervals between visits of the same bird using the recorded feeder data. The data were screened for errors as described above and the final data set consisted of 4804 records of visits to feeders and 2456 records of visits to the drinkers by the 10 birds.

2.3.3 Data Set 3

The third data set, as described in Yeates et al (2001), was used for testing the suitability of the new models against an independent data set. In summary, visits to feeders were recorded for 16 lactating cows that had access to 12 feeders, half of which contained a high protein feed and half a low protein feed. Each feeder consisted of a bin, accessed by a pneumatically controlled gate and cows were identified by a transponder worn around the neck. Cow number, start time of visit and weight of bin were recorded on entry of the cow and end time and weight were

recorded when the cow left the feeder. The feeder gate remained shut for 10s after the cow left to allow stabilisation of the feed bin. Time was measured to the nearest second and weight to the nearest 0.1 kg. Access to the feeders was continuous except for around 40 minutes during milking (twice daily) and between 08:00 and 09:30 when the bins were cleaned and restocked with fresh feed. Three-quarters of the feed was offered in the morning, and the rest was added to the bins during the afternoon milking. Water was available *ad libitum* from two troughs near each end of the row of feeders. Cows were housed in a yard at the Langhill Dairy Cattle Research Centre, Edinburgh for the duration of the experiment. Housing and management conditions were kept constant for the duration of the experimental period. A total of 79386 records were obtained from 16 cows during an average of 156.6 days per cow.

2.3.4 The Methods

2.3.4.1 Method 1

For the first method used to estimate meal criteria, the models developed by Yeates et al (2001) were adapted and a log-normal function was used to model the between-meal interval length distribution. Throughout this study, log refers to the natural log. The within-meal interval lengths were represented by a truncated log-normal distribution, with the truncation at 150 s (5 log units). Equation 1 shows the probability density function (PDF) for the overall model:

$$\begin{aligned} PDF = p\,(1/cunormal((5-\mu_1)/\sigma_1))\,(1/(2.506628\,\sigma_1))\exp(-((x-\mu_1)^2)/(2\sigma_1^2)) + \\ (1-p)\,(1/(2.506628\,\sigma_2))\exp(-((x-\mu_2)^2)/(2\sigma_2^2)) \end{aligned} \quad (1)$$

Where: p = proportion of data represented by the distribution of short interval lengths (within-meal), cunormal=GENSTAT cumulative normal correction factor to allow for truncation, μ_1,

σ_1 and μ_2, σ_2 = mean and standard deviation of the truncated log-normal distribution and log-normal distributions respectively, x = log transformed interval length in seconds

Model fitting was carried out using the maximum likelihood fit non-linear procedure in GENSTAT (VSN International 2008). The meal criterion was estimated at the interval length where the two distributions intersected. As this cannot be determined algebraically, the value was estimated to three decimal places using an iterative FORTRAN program.

2.3.4.2 Method 2

Method 2 used a single truncated log-normal function, with a truncation at 7.5 log units (corresponding to 30 min) to model part of the distribution of between-meal interval lengths. Equation 2 shows the PDF for this truncated log-normal:

$$PDF = (1/cunormal((7.5-\mu)/\sigma))(1/(2.506628\ \sigma))exp(-((x-\mu)^2)/(2\sigma^2)) \quad (2)$$

Where: cunormal = GENSTAT cumulative normal correction factor to allow for truncation, μ and σ = mean and standard deviation of the between-meal interval lengths distribution and x = log transformed interval length in seconds

By comparing the frequency distribution of the observed interval lengths with a PDF generated from the parameters of the fitted model, the meal criterion was determined as the point at which the PDF accounted for exactly half of the observed frequency.

2.3.4.3 Method 3

This model is based on analysis of the probability of animals starting to feed in relation to time since the last visit (Pstart). The observed probability of animals starting to feed within the next minute at time t since the last visit can be calculated from the observed data as the number of intervals > t and ≤ t +1 min divided by the number of intervals > t min. The meal criterion was estimated at the minimum point when Pstart was plotted against interval length. This value was determined, after visual inspection, by estimation of the point at which the difference in Pstart changed from being negative to positive, using a rolling average over 5 minute intervals to reduce the effect of random variation in values.

2.3.5 Effects of pooling

Preliminary analysis of data set 1 showed that STFB was not uniform across ages or between individuals using different feeding strategies. I therefore estimated meal criteria and associated meal characteristics both by week of age and by feeding strategy, and when pooled. Feeding strategies were investigated by counting each individual's average daily number of intervals > 30 min as these were assumed to be all intervals between meals. Because the number of observations collected per individual per week was too low to estimate accurate individual meal criteria, sets of 100 randomly selected birds each with a low (5 to 7), a near average (8 to 10) or a high (11 to 13) mean number of long intervals per day were used to study effects of feeding strategy. These groups represented birds with "few", "average" and "many" meals per day. The data set was, therefore, split in nine subsets by week of age as well as by feeding strategy to investigate the influence of pooling. Starting probability graphs were determined for each of these groups and on the basis of these graphs, and truncated Weibull distributions

(truncation at 7.5 log units, corresponding to 30 min) were fitted to the frequency distribution of log-transformed interval lengths. The PDF was:

$$((1/(1-\exp(-(7.5b)^a)))*((ax^{(a-1)})*(\exp(-((x/b)^a)))/(b^a)) \quad (3)$$

Where: a=Weibull shape parameter, b=Weibull scale parameter, x = log transformed interval length in seconds

Once the best-fitting truncated Weibull had been established using the fit non-linear procedure in GENSTAT, the meal criterion was derived in the same was as in Method 2 and the average number of meals per day for each group was calculated. These values were then compared to those found using the pooled meal criterion on the same data subsets using a paired t-test.

2.3.6 Testing against an independent data set

Instead of method 1, the original method from Yeates et al (2001) was compared with methods 2 and 3 for their suitability for estimation of meal criteria in cows. In all cases, the meal criterion was derived using the pooled data set. These values and the meal characteristics were then compared to the values found by Yeates and colleagues using their 2 - or 3 -process models (2001). Differences between meal characteristics estimated according to the different methods were then analysed using two-way ANOVA analysis.

2.4 Results and Discussion

2.4.1 Validation of feed station technology

Figure 1 shows the comparison between the amount of feed eaten per day and the amount of feed recorded by the electronic feed station. Each of these values was totalled across the 8 feed places in the feed station. The graph shows that the feed stations recorded over 98% of the actual feed eaten and assigned it to a bird, with some days reaching 100% accuracy. It was therefore concluded that the scales were accurately recording the correct amount of feed eaten by each bird.

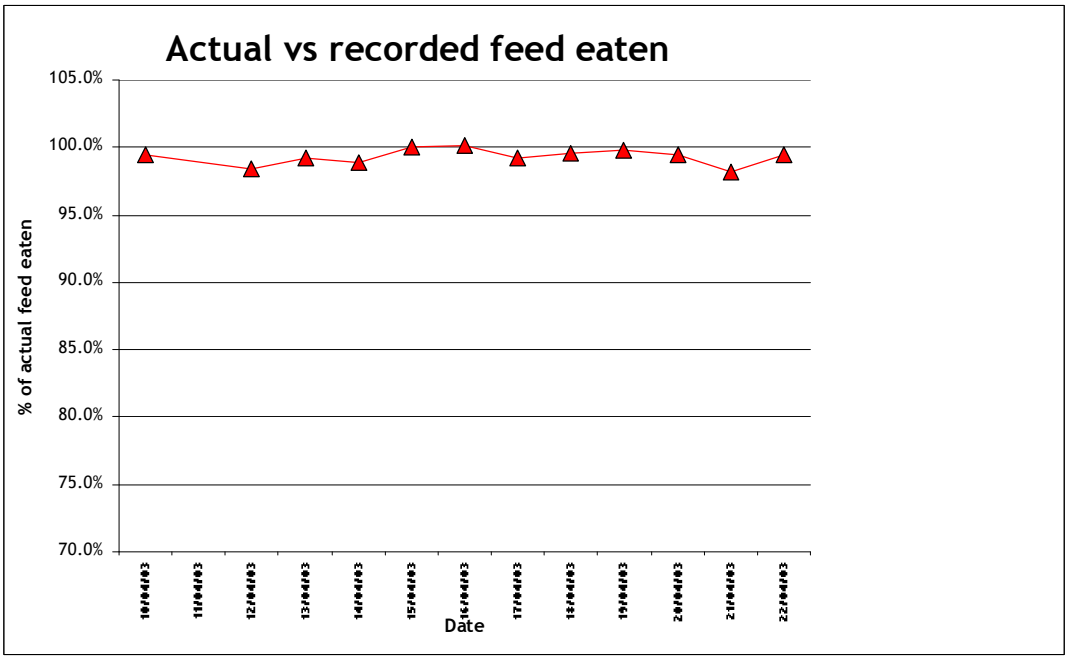

Figure 1: Comparison between feed intake recorded by the feed stations and actual feed intake

To confirm that the electronic system was correctly identifying the bird which was feeding, data from dataset 2 on coloured birds was used, comparing the observed visits to feeders by these birds with the data recorded by the feed stations over the 3 week period. All visits of

these birds to the feeders were correctly identified by the system, and it was therefore concluded that the data from the feed stations was measuring feeding behaviour accurately.

2.4.2 Summary of Data

Table 1 shows a summary of the main characteristics of data set 1. There was a large increase in the size of the birds during the experimental period, which is reflected by the nearly five-fold increase in body weight from start day (474g, SD = 56.5g) to end day (2275g, SD = 174g).

Table 1: Means (± standard error) of feeder occupancy, number of daily visits per bird and daily feed intake of broiler birds aged between 2 and 3, 3 and 4 and 4 and 5 weeks.

	Mean week 2-3	Mean week 3-4	Mean week 4-5
Feeder occupancy (%)	58.23 ± 3.88	69.5 ± 1.18	73.5 ± 3.33
Number of visits per bird	34.5 ± 0.33	35.1 ± 0.31	32.3 ± 0.29
Daily intake (g)	107 ± 0.33	150 ± 0.28	172 ± 0.38

Figure 2a shows a histogram of the interval lengths between visits of data set 1.With increasing interval length, the frequency decreases rapidly, reaching a minimum at approximately 900s, then starts to increase to a peak at around 3000s followed by a gradual decline to very low frequencies at long intervals. This is similar to the distribution seen in cows (Tolkamp et al., 1998) and ducks (Bley 2005). For ease of model fitting, the data were log transformed, as suggested by Tolkamp et al (1998), leading to the distribution seen in Figure 1b. Because the probability of animals starting to feed is lower during dark than during light periods (e.g. Morgan et al., 2000b), there was an elongation in intervals over the dark period showing as a small blip in the graph around 9.5 log units (3.7 hours). These intervals (~5% of total) were

removed for the purpose of fitting the model to estimate meal criteria (Figure 2c) but later included for the calculation of meal characteristics.

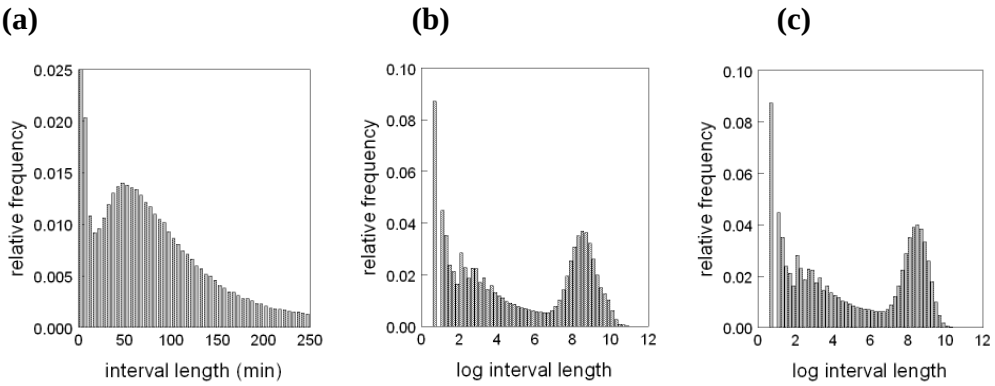

Figure 2: Histograms of interval length between visits. Graphs (a) and (b) show the distributions of interval lengths in min and the intervals lengths (measured in s) after log-transformation, respectively. Graph (b) shows a small blip in the distribution around 9.5 log units (3.7 hours). Graph (c) shows that this blip disappears in the distribution of log-transformed intervals lengths after intervals that include the dark period have been removed. The bin width is 200s for the non-transformed data and 0.2 $\log_e$ units for the log graphs. As the intervals are calculated to the nearest second, there are some empty bins initially in graphs (b) and (c) as there is no data between 1 and 2s, for example.

Initial attempts to fit several two- or three-population models consisting of combinations of Weibull, normal and negative exponential to data set 1 did not result in an adequate description of the distribution of log-transformed interval lengths as the within-meal interval length distribution could not be suitably represented by any of the tested combinations of distributions. Hence existing models had to be adapted for use with these data.

2.4.3 Method 1, Data Set 1

It has been observed in several species (Tolkamp and Kyriazakis 1999a; Zorrilla et al., 2005) that there may be a population of relatively long within-meal intervals during which animals drink and that a log-normal may give an appropriate description of such populations (Yeates et al., 2001). I hypothesised that birds would similarly produce such a population of intervals. In addition, I hypothesised that it would be likely that the vast majority of within-meal intervals longer than 150 s would be intervals during which birds visited the drinker. A mixed model including a log-normal for the longer intervals and a truncated (at 150 s) normal for the shorter intervals was, therefore, fitted to the observed data. Truncated log-normal models have been used successfully in the past to describe STFB of cows (DeVries et al., 2003). This model seemed to give a good fit to the observations (Figure 3a). A log-normal was used for the pooled between-meal interval length population as it gave a better fit than the Weibull (as used by Yeates et al., 2001) using the GENSTAT maximum log-likelihood procedure, and the Pstart for the data set followed the distribution expected for a log-normal rather than a Weibull (Figure 4a). The point at which the two distributions intercept was calculated at 1252 s and this was initially thought to represent an appropriate meal criterion. This is slightly shorter than the meal criterion found by using similar methods in cows (Tolkamp et al., 1998; Tolkamp et al., 2000). Previous studies on birds used either an arbitrary meal criterion or one estimated by the log-survivorship method, resulting in much shorter meal criteria of between 1 and 2 min (Duncan et al., 1970; Masic et al., 1974; Wolf and Hainsworth 1977; Savory 1980). As these meal criteria values are so different due to the technique used for estimation, results from these studies cannot easily be compared to those from the present one.

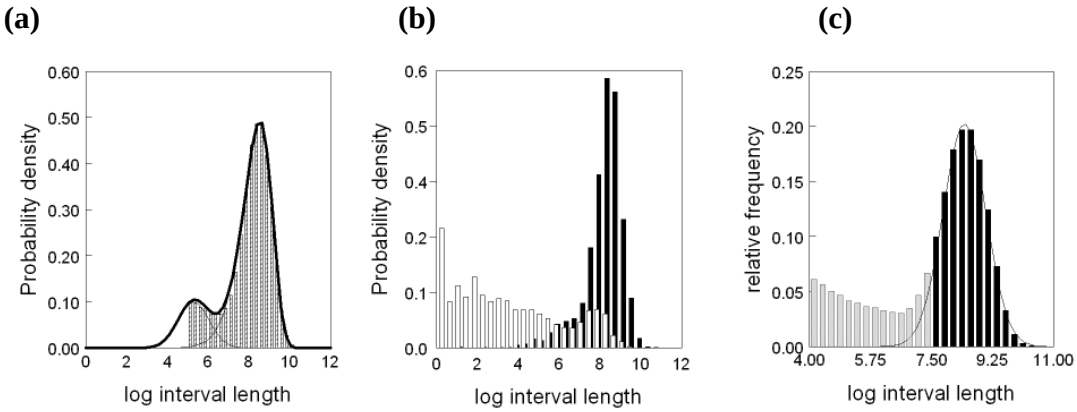

Figure 3: (a) Method 1, data set 1; fit of the truncated log-normal and log-normal distributions (curves) to the observations (bars, which are the relative frequency of log-transformed interval lengths divided by bin width = 0.25 log units). The bold line shows the fit of the sum of the two distributions to the data, whereas the thinner lines show each of the two distributions separately. The point at which these two distributions intercept represents the meal criterion which was calculated by iteration. **(b) Data set 2; observed probability density (bars, which are the frequency distribution of log-transformed interval lengths divided by bin width= 0.5 log-units) of intervals with (black bars) or without (white bars) drinking bouts (c) Method 2, data set 1; fit of the truncated normal** (curve) to the observations (bars, which are the frequency distribution divided by bin width = 0.25 log units) of which only the data represented by the dark bars were used to fit the truncated log-normal.

2.4.4 Method 1, Data Set 2

The meal criterion estimated by method 1 rested on the assumption that the longest within-meal intervals were due to animals leaving the feeder to drink. The feeding behaviour of birds in data set 2 (in terms of the distribution of intervals between visits, number of visits and visit size) was similar to that in data set 1. This makes the observations of drinking behaviour seen here relevant for the interpretation of the frequency distribution of intervals in data set 1.

Figure 3b shows the frequency distribution of intervals during which the chicks did (black bars) or did not (white bars) drink. Truncation was applied at 150 s as it was hypothesised that within-meal intervals longer than this would mainly consist of intervals that contained drinking bouts. As the drinkers were situated at 0.8 m and 1.5 m from the feeders, I estimated it would not take more than this length of time for a bird to leave a feeder, drink and return. The data in Figure 3b show that the assumption that the truncated normal distribution in method 1 representing within-meal drinking intervals is incorrect, as there is no evidence of a significant distribution of intervals containing drinking within-meals. Even though Tolkamp and Kyriazakis (1999b) and Yeates et al (2001) found a three-process model incorporating drinking as ideal for modelling the feeding behaviour of cows, there were some individuals that did not drink during meals. There is little evidence in the literature of socially housed birds routinely drinking within meals (unlike in rats and pigs - e.g. Kissileff 1970; Morgan et al., 2000a). There is also very little within-meal drinking occurring in the birds in this study. As a consequence, most of the drinking activity occurred during the longer (between-meal) intervals. The use of model 1 cannot be justified, therefore, on the assumption that there is a distribution of longer intervals within meals during which birds drink.

2.4.5 Method 2

As an alternative to method 1, I developed a second model for situations when the appropriate between-meal interval length distribution is known but the within-meal distribution is not. In general, the meal criterion that minimizes the number of mis-assigned intervals is the value at which the PDF of the model describing the distribution of between meal intervals is equal to the PDF describing the distribution of within-meal intervals (Slater and Lester 1982). By definition, this meal criterion value occurs at the interval length where the combined interval

frequency is twice the frequency predicted by the between-meal interval distribution only. If a PDF describing the distribution of between-meal intervals can be fitted, a meal criterion can be estimated at the interval length at which the observed frequency distribution is twice the value predicted by this PDF.

Figure 3c shows the fit of the truncated log-normal model to intervals greater than 7.5 log units. Truncation was made at this value because intervals longer than 1800 s were unlikely to be part of the frequency distribution of within-meal interval lengths. A meal criterion of 1203 s was estimated from the model parameters. This method uses the same techniques as in method 1, but has the advantage of not requiring identification of suitable within-meal interval length distributions, nor any assumptions about drinking.

2.4.6 Method 3

Method 2 can only be applied if a suitable between-meal distribution can be identified. A third method was developed for situations where neither the within- nor the between-meal interval distributions are known. Pstart, calculated on the basis of all intervals combined, decreases rapidly at short intervals and then shows an increase from approximately 1000 s (Figure 4a). For long intervals, the calculated Pstart is determined by a single population of intervals (the between-meal intervals) and it is expected that Pstart will increase with time since the last meal as a result of satiety wearing off (Yeates et al., 2001). At short interval lengths, however, the value of Pstart is determined by a mixture of the Pstart associated with the population of within-meal intervals and Pstart associated with the population of between-meal intervals. Since Pstart within a meal is very much higher than Pstart between meals (Slater and Lester 1982; Tolkamp et al., 2002), an increase in interval length at short intervals will result in a

decrease in Pstart. The decrease in Pstart will reach a nadir and at longer interval lengths, when Pstart is being dominated by intervals between meals, will start to increase again. It can therefore be argued that when Pstart decreases with increasing interval length, intervals are dominated by within-meal intervals and when Pstart increases, it is the between-meal intervals which dominate. Therefore the nadir will give a good approximation of the meal criterion if the form of interval distributions is unknown. Visual estimates of the nadir in starting probability for different hatches were between 900 and 1170 s. The calculated change in starting probability switched from negative to positive at 1050 s, which was assumed to represent the meal criterion. This estimate is nearly 200 s less than that given by methods 1 and 2. No models incorporating starting probability have been used before to estimate meal criteria, although the concept has been previously discussed (Tolkamp et al., 1998; Morgan et al., 2000b; Yeates et al., 2001). Although this model may not be as robust for meal criterion determination as method 2, it could be useful for situations when neither the within- or between-meal interval length distributions are known, depending on the effects of the difference in estimates on meal characteristics (see below).

As can be seen in Figure 4a, the starting probability levels off and then begins to decrease from around 10,000 s (2 h 45 min), instead of continuing to increase, which would be expected from the concept of satiety (Yeates et al., 2001). As this was unexpected, I investigated whether it was due to an effect of pooling (Morgan et al., 2000b).

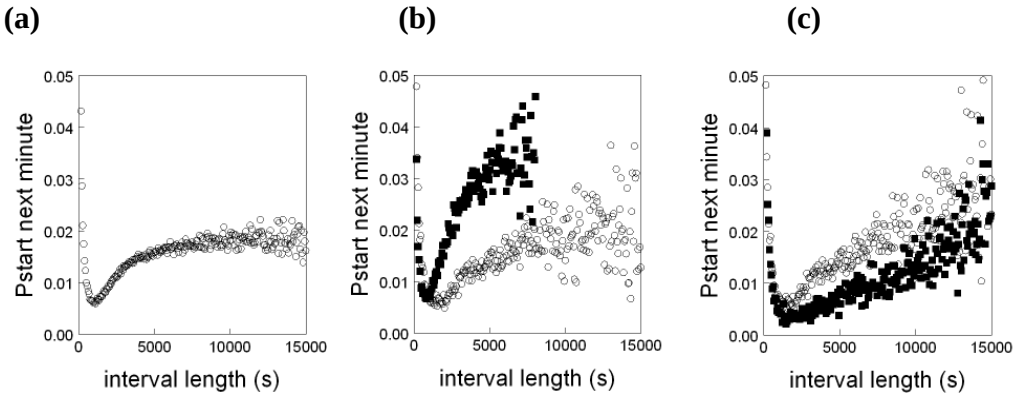

Figure 4: Method 3, data set 1; (a) probability of birds starting to feed (Pstart) within the next minute against time since the last visit for the pooled data. (b) Pstart against time since the last visit for birds with many meals at either 3 weeks (■) or 5 weeks (○) of age to illustrate effects of disaggregation by age. **(c) Pstart against time since last visit for birds aged 5 weeks** with either few (■) or many (○) meals per day, to illustrate effects of disaggregation by feeding strategy. Only data points based on more than 100 observations were plotted in any graph.

2.4.7 Effects of the different methods on estimated meal characteristics in birds

2.4.7.1 Effect of pooling

The effect of using a pooled meal criterion on estimates of number of meals per day compared with using individual meal criteria for each age-feeding strategy group was assessed for method 2, because when tested on data from other species, this method was found to give the most similar meal criterion estimate to previous models. This time, a truncated Weibull was used to estimate the meal criteria instead of a normal as the Pstart continued to increase with time, unlike that from the pooled data. This is in accordance with the predictions of a Weibull distribution. Pstart was calculated for each of the nine age feeding strategy sub-groups. Pstart did continue to rise after the nadir at around 1000 s in each of the sub-groups. As younger birds

have many more visits to the feeder than the older birds, and therefore shorter between-meal intervals, Pstart calculated from the pooled data is dominated at shorter interval lengths by data from these younger birds but by data from older birds at longer interval lengths. As the starting probability is generally lower in older birds, this change from the Pstart being dominated by younger to being dominated by older birds creates an artificial decrease in Pstart at longer intervals when all ages of birds are pooled (Figure 4a). However if the Pstart of young and older birds are considered separately, the expected continual rise in Pstart between meals can be observed (Figure 4b). This is also the case with different feeding strategies of birds, as birds with many meals tend to have fewer long intervals between feeding visits than birds with few meals (Figure 4c). The observed continuous increase in Pstart with time shown by the age-feeding strategy groups is in accordance with the concept that feeding behaviour in the short-term is controlled by satiety in these birds as is the case in other animals.

Figure 5 shows that only the group with many visits to the feeder in the first week of the experiment showed a significant ($p<0.01$) difference in meal number, based on the pooled versus the sub-group estimation of meal criteria. This may be due to pooling across days within the week as the birds grow rapidly at this age. Evidence for this comes from the decline in Pstart at longer intervals shown by this group (Figure 4b). The pooled meal criterion was higher than any of the sub-group estimates and, as a result, the estimates for number of meals per day are universally lower if they are based on the former rather than the latter meal criterion. Because the differences in meal number were either non-significant or very small, I concluded that a meal criterion estimate based on the pooled data set would still be appropriate.

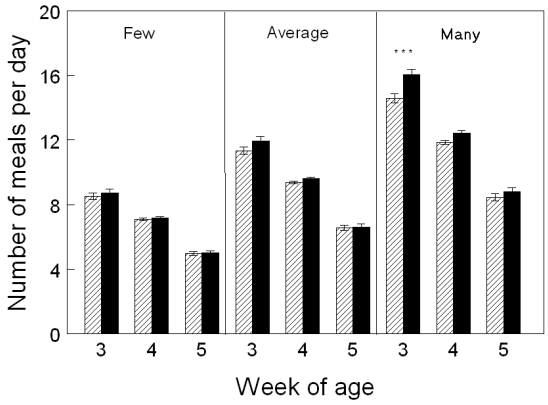

Figure 5: Predicted mean (± standard errors) number of meals based on meal criteria estimates obtained by fitting a truncated Weibull to data disaggregated by age (3, 4 or 5 weeks) and feeding strategy (few, average or many meals per day) as black bars and predicted number of meals based on a single meal criterion estimated by fitting a truncated normal model to the pooled data (as dashed bars).

2.4.7.2 Effects of different methods

Table 2 shows the estimates for meal characteristics that were derived from the meal criteria estimated from the pooled data by each of the 3 methods. Means and standard errors for each characteristic were calculated for the pooled data set.

Table 2: Means (± standard error) of individual meal characteristics of individual broiler birds in data set 1, derived after grouping visits into meals on the basis of meal criteria estimated with method 1 (log-normal with truncated log-normal), method 2 (truncated log-normal) and method 3 (changes in Pstart).

	Method 1	Method 2	Method 3	ANOVA analysis
Meal criterion (s)	1252	1203	1050	
Meals per day	10.6 ± 0.08	10.6 ± 0.08	10.7 ± 0.08	$F_{2,1057}$ = 1010.9 ***
No. visits/meal	3.01 ± 0.04	3.00 ± 0.04	2.96 ± 0.04	$F_{2,1057}$ = 950.0 ***
Meal duration (min)	8.06 ± 0.09	7.93 ± 0.09	7.61 ± 0.09	$F_{2,1057}$ = 1348.0 ***
Intake per meal (g)	13.3 ± 0.10	13.2 ± 0.10	13.1 ± 0.10	$F_{2,1057}$ = 1379.2 ***

All characteristics were found to be statistically significantly different at the $p<0.001$ level using a two-way ANOVA analysis. However, the actual difference between the values on a practical level of interpreting feeding behaviour is incredibly small and with around 1000 data points, any slight variation is likely to result in a significant difference. Also, as the two-way ANOVA was conducted on within-flock data using a meal criterion derived from pooled data, the effect of meal criterion on the meal characteristics was always in the same direction for all animals, making the results statistically significant. However, there was still only a very small difference between the methods, as illustrated by the fact that around 80% of the birds had a less than 2.5% change in number of meals per day when the two methods with the most different meal criteria (methods 1 and 3) were compared. Therefore I conclude that the three tested methods do result in slightly different meal criteria estimates but that, as a result of the scarcity of intervals in the 1000 to 1300 s range this has very little effect on the resulting meal characteristics.

2.4.8 Testing of the Methods against Independent Data obtained with Cows

The two successful new methods (the second and third) for estimating meal criteria were applied to the data set obtained with cows that were previously analysed by Yeates et al (2001). The resulting meal criteria, and their effects on meal characteristics, were compared with those based on the double log-normal and log-Weibull model that Yeates et al (2001) identified as most appropriate for estimating meal criteria for that data set. Table 3 shows the meal characteristics derived for each of the models.

Table 3: Means (± standard errors) of meal characteristics calculated from data obtained with 16 cows, based on meal criteria estimated with Method Yeates (according to Yeates et al 2001, based on two log-normals and a log-Weibull), Method 2 (one truncated Weibull, this chapter) and Method 3 (changes in Pstart, this chapter).

	Method Yeates	**Method 2**	**Method 3**	**ANOVA analysis**
Meal criterion (s)	1734	1676	2128	
Meals per day	5.99 ± 0.20	6.00 ± 0.20	5.90 ± 0.19	$F_{2,13}$ = 121.33 ***
No. visits/meal	5.35 ± 0.37	5.33 ± 0.37	5.43 ± 0.37	$F_{2,13}$ = 86.17 ***
Meal duration (min)	31.7 ± 1.25	31.6 ± 1.24	32.7 ± 1.31	$F_{2,13}$ =103.25 ***
Intake per meal (kg)	7.40 ± 0.28	7.42 ± 0.28	7.54 ± 0.28	$F_{2,13}$ = 109.86 ***

From Table 3 it is obvious that the meal characteristics derived from the meal criteria estimated by the three methods differ only very little. However, ANOVA tests revealed significant differences between all the meal characteristics for the same reasons as described above for the chicken data. These may be statistically significant but, like the chicken data, the actual differences were trivial. Surprisingly, the third method resulted in a longer estimated meal

criterion compared to method 2 when applied to the cow data but a shorter one when applied to the bird data. As can be seen from Table 3, the methods may not give exactly the same meal characteristics but the estimates are affected very little by the selected method. This is a result of the scarcity of intervals in the range of 1500 to 3000 s. This shows that the methods developed here on the basis of bird data give similar estimates of the meal criterion as a method that was specifically developed for analysis of cow data.

2.5 General Discussion

Daily feed intake is determined by meal size and meal frequency (Meguid et al., 1998; Emmans and Kyriazakis 2001). The relationship between the two can provide answers about how feed intake is regulated on a daily basis (Tolkamp and Kyriazakis 1999a). One of the main reasons that meal pattern analysis has stagnated is that there has been little unanimity as to the appropriate definition of meals (Geary 2005).Yet, an appropriate definition of meals is crucial to avoid biased conclusions from meal pattern analysis. For example, Zorrilla and colleagues (2005) found that the use of one meal criterion, estimated using conventional log-survivorship methods suggested feed deprivation in rats resulted in a subsequent increase in meal frequency but no change in meal size. In contrast, the use of a meal criterion estimated by a method derived in their paper, lead to the conclusion that following chronic feed deprivation rats increased meal size but not frequency (Zorrilla et al., 2005). This shows how important it is to determine a biologically relevant meal criterion before grouping feeding events into meals, to avoid drawing the wrong conclusions from analyses of short-term feeding behaviour. Researchers in the past have used a wide range of meal criteria for, e.g., cattle (from 2 min to more than 40 min: Forbes 1985; Harb et al., 1985; Stamer et al., 1997) and rats (from less than 2 min to 40 min - Lemagnen and Tallon 1963; Zorrilla et al., 2005), thus making comparisons in meal patterns from different studies impossible. Many of the meal criteria used in the past were arbitrary or based on models with un-biological underlying assumptions (Tolkamp et al., 1998). Some recently developed models are based on the satiety concept and rely on analysis of recognisable distributions of interval lengths (Tolkamp and Kyriazakis 1999b; Yeates et al., 2001).The models proposed in this chapter allow for the estimation of meal criteria even when the most appropriate interval distributions are not easily identifiable. These methods estimate meal criteria independently from their effects on meal duration and meal size and they differ

favourably in this respect from the method proposed by Zorrilla et al (2005). The successful application of the methods developed with bird data to observations collected with cows suggests that these can be applied across species.

Whereas many studies in the past have relied on a small number of observations, I used data obtained with a total of 1058 birds over a period of 21 days and had over 700,000 visit records available for analysis. This large data set allowed us to do very detailed analyses of the different methods, of the changes in starting probability and of the effects of pooling across age and feeding strategy on Pstart, meal criterion estimates and meal characteristics. At the same time, the large number of data led to the finding that very small differences in meal characteristics that resulted from different methods to estimate meal criteria sometimes attained statistical significance. This illustrates that trivial differences from a biological point of view can still attain statistical significance if the number of observations is large enough.

Pooling of data across age and of birds with different feeding strategies can obscure effects of time since the last meal on the probability of an individual starting a meal at any given age and, therefore, lead to wrong conclusions about the mechanisms underlying short-term feeding behaviour. For the investigation of such mechanisms, therefore, disaggregation of the data may well be required. The analyses show, however, that data pooling before estimation of meal criteria has very little effect on the resulting meal characteristics. Cumbersome estimation of meal criteria for each age and/or feeding strategy class may, therefore, not be required, which makes the methods much more user friendly.

In conclusion, the new methods developed here can be useful tools in short-term feeding behaviour analysis when the data do not show clearly identifiable distributions of within- and/or between-meal interval length frequencies and these analyses show that they can be applied for meal criteria estimation in species as different as young growing birds and large lactating ruminants. The resultant estimates of meal characteristics can then be used for the purposes of the various issues I have raised above.

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CHAPTER 3

The structure of feeding behaviour in commercial broiler lines selected for different growth rates

Adapted for publication in Poultry Science: 88 (6): 1143-1150

Chapter 3 – Structure of feeding behaviour in four broiler lines

3.1 Abstract

Selection for increased growth rate in livestock is accompanied by increased requirements for food resources. It has been suggested that more intensively selected birds, such as broilers, have altered feed intake control mechanisms and may be constantly hungry, due to the high demands of fast growth rates. If this is the case, it would be a major welfare issue. I investigated the hypothesis that more intensive selection for growth in some lines of broilers has altered feeding behaviour by analyzing short-term feeding behaviour bouts in relation to the roles of hunger and satiety mechanisms in the control of feed intake. Using four genetic lines, resulting from different levels of selection for growth rate, meal pattern analysis was performed and the bouting of short-term feeding behaviour estimated. All lines showed bouted feeding behaviour, although differences in meal size, number of meals and meal duration were evident across lines. In all lines, the probability of animals starting a new meal was low immediately after finishing the previous meal and increased with time, as expected for feeding behaviour governed by hunger and satiety mechanisms. Normal feeding behaviour was, therefore, not affected by the intensity of selection. Feeding rate increased with growth rate, suggesting that this may be a consequence of selection. However the other characteristics of feeding behaviour, such as meal duration, did not change consistently with higher growth rate. Due to differences between lines in bird size, the number and weight of birds per pen also differed between the lines. The differences in feeding behaviour between lines were greatly diminished when weight of birds per m^2 was taken into account but were still statistically significant. Overall, it is apparent that even when growth rate and body size have been substantially altered by genetic selection, the underlying normal controls of feeding behaviour are conserved in broiler birds.

3.2 Introduction

Over the past 50 years, the livestock industry has seen rapid advances in the growth rate of animals aided by genetic selection for desirable traits. The largest changes have been seen in broilers (Barbato 1994), with birds now achieving a slaughter weight of more than 2.2 kg in just 42 days (Bokkers et al., 2004). With these rapid growth rates come an associated increased demand for food resources, so average daily intakes are higher in these faster growing birds compared to slow growing birds of the same weight or age (Emmans and Kyriazakis 2001). It has been suggested that selection has resulted not only in increased appetite (Denbow et al., 1986; Dunnington and Siegel 1996), but that these birds also no longer exhibit normal feeding behaviour and are constantly hungry (Burkhart et al., 1983; Bokkers et al., 2004). If this is the case, this would be a major welfare issue (Farm Animal Welfare Council 1992).

Feed consumption generally occurs in short feeding events, such as visits to a feeder, which are clustered into "meals". Meals are considered to be a more biologically relevant unit in which to study short-term feeding behaviour (STFB) than feeding events such as visits (Tolkamp et al., 2000). Meal size and duration are thought to be controlled by hunger and satiety mechanisms that affect the start and termination of meals, whereas visit characteristics are more subject to random events. Satiety, associated with the end of meals, will result in a low probability of animals starting a meal (Pstart) immediately after finishing one. As satiety decreases, Pstart is expected to increase with time since the last meal. This increase in Pstart results in a characteristic distribution of between-meal interval lengths that can be clearly distinguished from the distribution of within-meal interval lengths (Tolkamp et al., 1998). The clustering of feeding events into meals, the characteristic distribution of between-meal intervals and the

increase in Pstart with time since the last meal are, therefore, part of the typical expression of normal feeding behaviour of *ad libitum* fed animals.

Any alteration in the feed intake control mechanisms as a possible correlated response to selection for growth will lead to changes in the patterns of bouting (i.e. the clustering of visits into meals). The first aim of this chapter was to test whether the degree of bouting of STFB, and consequently feed intake control mechanisms, have been altered by different intensities of selection in modern broiler lines. In addition, I aimed at testing effects of this genetic selection on the probability of animals starting to feed in relation to time since the last meal (Pstart). STFB data were collected using electronic feeders that registered visits to feeders by individually identifiable birds. It was hypothesised that the extent of any effect on the structure of STFB, in terms of bouting of behaviour and changes in Pstart with time since the last meal, would be correlated with the degree of selection intensity, i.e. birds resulting from more intensive selection for growth rate were expected to have a larger deviation from normal feeding patterns than those with a lower growth rate. I tested this hypothesis by investigating STFB of four genetic lines of broiler birds, which differed in their degree of selection for growth rate.

3.3 Materials and methods

3.3.1 Animals and Housing

The experimental set up for data collection was similar to that described in Howie et al (2009). Feeding behaviour data were collected from a total of 16,283 female birds aged between 2 and 5 weeks from four genetic lines. Line A had been selected most intensively for growth, achieving an average of 2.4 kg at age 35 days, followed by lines B (2.1 kg at 35 days), C (1.9 kg at 35 days) and D (1.6 kg at 35 days). Data were available for 3,470 birds for line A; 4,257 for line B; 4,153 for line C and 4,403 for line D. Data were analysed for twelve hatches of each line, one per calendar month. Birds of different lines were housed together in the same sheds, with each hatch of each line consisting of three pens of birds of the same line (3.0m x 2.7m). Every pen contained two bell drinkers, eight electronic feeders (Beck Plastics, Cumbernauld, UK), similar to those described by Bley and Bessei (2008), and a layer of wood shavings covering the floor, which was topped up weekly. Numbers of birds per pen varied slightly between lines to take account of weight differences and the resulting mean number of birds per pen across the lines was 116. This gave a density of approximately 14.5 birds per feeder, which was considered to be sufficient as the feeder occupancy throughout the trial was less than 70% during the light hours, thus giving the birds ample opportunity to feed. The number of birds per pen also varied slightly within lines, which reflected the variation in the number of chicks available per hatch. Chicks were placed immediately into the pens on leaving the hatchery but no data were recorded until day 14, the start of the experimental period. This allowed for adaptation of the birds into the experimental conditions and feeders. During this period, temperature was gradually reduced from 27 ^{o}C to 19 ^{o}C in line with commercial husbandry practice, and the ratio of number of hours light to dark was kept constant at 20:4. Birds were weighed at the beginning and end of the 3 week experimental period.

All birds were fed a standard commercially available pelleted diet throughout the experimental period containing 210 g Crude Protein and 13.3 MJ Metabolisable Energy per kg feed. This diet was the recommended wheat-based grower diet for Ross 308 birds and details of the feed composition are shown in Table 1 in the appendix. Feed was available *ad libitum* from the electronic feeders, with only one bird able to enter a feeder at a time. Approximately 20g of feed was available from the feed dish of each feeder at any one time, which was automatically refilled from large feed hoppers. Birds were identified by means of a small wing band transponder which transmitted the unique ID code to the computerized recording system when a bird entered and left a feeder, using a radio frequency antenna system. Previous observations suggested that the presence of the wing band did not affect the behaviour of the birds (Howie et al., 2009). Scales connected to the feeders automatically recorded the start and end weight of feed for each visit and sent this information to the central computer. These data were stored together with the date, start and end time of the visit, and bird, feeder, pen, shed and farm ID codes. Resolution of the feeder clock was to the nearest 1 s and the scales were to the nearest 0.1 g.

3.3.2 Data Manipulation

The data were screened to remove birds which did not complete the experiment as well as inaccuracies from the electronic data recording system. If the wing band was not read correctly then the visits were excluded from further analysis (~0.2% of all visits). If the recorded weight of feed consumed was less than -0.1g (<0.5% of all visits), then the data were excluded from calculation of meal size but used for the other analyses. The intervals between all subsequent visits by the same bird were calculated and used for establishing a meal criterion to group the visits into meals. As the within-meal interval length distribution(s) could not be identified but

the between-meal interval distribution could, the methodology described in Howie et al.,(2009) was used for estimating the meal criterion. In brief, the interval lengths between visits to the feeders for all birds were log-transformed ($\log_e$) and a truncated log-normal model was fitted per hatch to the intervals lengths longer than 1800 s (corresponding to 7.5 log units), using a maximum log-likelihood approach in GENSTAT (VSN International, 2008). Equation 1 gives the model.

$$PDF = (1/\Phi\ ((7.5-\mu)/\sigma))(1/(2.506628\ \sigma))\exp(-((x-\mu)^2)/(2\sigma^2)) \quad (1)$$

Where: "PDF" is probability density function; Φ is GENSTAT cumulative normal correction factor for truncation at 7.5 log units; μ and σ are mean and standard deviation of the distribution of x, i.e. log-transformed interval length between visits (in seconds).

A meal criterion was estimated for each hatch at the point at which the normal model accounted for exactly half of the observed frequency of log-transformed interval lengths, as discussed in Howie et al (2009). The mean meal criterion estimate was then calculated per line as the mean of the 12 hatch estimates.

The probability of birds starting to feed within the next minute was calculated from the observed data at interval length t as: the number of intervals > t and ≤ t +1 min divided by the number of intervals > t min (Tolkamp and Kyriazakis 1999). The change in Pstart between meals was then calculated as the difference in Pstart between the minimum point and the value at 60 min. To determine whether the degree to which feeding behaviour was bouted had been altered by selection, I analysed histograms (bin width 5 min) of interval lengths between visits.

The degree of bouting was determined by calculating the ratio between relative frequencies of the bin containing the calculated meal criterion and the bin containing the peak in the frequency of the long intervals (e.g. a ratio of 1.5 would indicate a peak with a relative frequency 1.5 times higher than the relative frequency at the meal criterion). This was done for individual hatches and a mean and error was estimated per line.

Once a meal criterion was estimated, meals were identified as clusters of visits that were separated by intervals shorter than the meal criterion, and separated from other meals by intervals longer than the meal criterion. Mean STFB characteristics were then calculated using data from individual birds. Traits analysed were: number of meals per day, meal duration, meal size (intake per meal), visits per meal, feeding rate and pause time within meals (total within-meal interval time). Also calculated were means of start and end body weights, body weight change and daily intake.

The effect of number of birds per m^2 on meal characteristics was analysed to establish whether this was influencing any differences found between lines. As the experiment was started for all the birds at the same age, body weight also varied between the lines thus the effect of weight of birds per m^2 was also estimated. This was calculated as a mean total weight (i.e. sum of start and end weight, divided by 2) for each pen and divided by the pen area.

3.3.3 Statistical analysis

In order to determine the statistical effects of genetic selection on bouting, meal characteristics and performance traits, one-way ANOVA was performed on data per hatch (bouting) and per bird (meal and performance traits) with line as a factor. Pstart differences between the lines

were compared using REML analysis, using line and time since last feed as fixed effects. The differences in change in Pstart between the nadir of the graph and 60 min since last feed were analysed using a one-way ANOVA. To determine the effects of number and mean weight of chicks per m^2 on meal characteristics, REML analyses were also performed, with number/weight of birds per m^2 nested within line as the fixed effects, and bird nested within pen within hatch as random effects. Number/weight of birds per m^2 was included as a fixed effect to account for the differences in stocking densities between the lines.

To assess the biological significance of any statistical significance found by analysis of weight of birds per m^2, the mean values per trait were calculated for each line at a mean weight per m^2. Once a mean weight per m^2 across all the lines had been determined, each meal characteristic was estimated for birds stocked at this density, using regression analysis. Regression lines of weight per m^2 against meal characteristic were fitted to the individual bird data per line and the parameters used to estimate each characteristic at the mean value across lines. All statistical analyses were performed using GENSTAT.

3.4 Results

3.4.1 Performance traits

Table 1 shows the differences in performance traits, intake per visit and total recorded number of intervals between visits to feeders for the four lines over the 3 week experimental period. Line A birds were indeed the heaviest at both the start and end of the period, and had the fastest growth rate and lowest feed conversion ratio (FCR). The other lines varied systematically in their performance characteristics with weight change, with line D having the lowest start weight, end weight and growth rate, and highest FCR. Intake per visit varied between the lines, with line A showing the highest and line C the lowest (Table 1). All differences between lines were statistically significant at the $p < 0.001$ level.

Table 1: Performance and visit-based traits for the four lines of birds across the 3 week experimental period. Mean (± standard error) weight at the beginning and end of the observation period are given, with feed conversion ratio calculated as the amount of feed consumed (g) divided by weight gain (g). n = number of birds ANOVA. F statistics are given for performance traits and intake per visit, all with degrees of freedom = 3, 16279.

	Line A (n = 3470)	**Line B (n = 4257)**	**Line C (n = 4153)**	**Line D (n = 4403)**	**ANOVA**
Start weight (g)	522 ± 0.85	494 ± 1.19	442 ± 0.66	420 ± 0.84	F = 2523 ***
End weight (g)	2350 ± 3.18	2100 ± 2.91	1890 ± 2.30	1660 ± 2.38	F = 11700 ***
Weight change (g)	1830 ± 2.76	1600 ± 2.72	1440 ± 1.96	1240 ± 2.12	F = 10490 ***
Feed conversion ratio	1.61 ± 0.002	1.63 ± 0.002	1.67 ± 0.001	1.72 ± 0.002	F = 623.1 ***
Intake per visit (g)	5.66 ± 0.04	5.33 ± 0.04	3.41 ± 0.02	3.76 ± 0.03	F = 1098 ***
Number of visit records	2138435	2681245	3308277	2959202	

3.4.2 Bout Analysis and Pstart

Figure 1 shows the distribution of the interval lengths between visits per line. There is a very high relative frequency in the first bin (intervals < 5 min), followed by a decrease in frequency with interval length to a minimum around 15 to 20 min. At intervals longer than this minimum, the frequency distribution rises with interval length to a peak at around 60 min and then falls again to very low values at very long intervals. Figure 1 shows that the distribution pattern of interval lengths between visits is very similar across the four lines, and all show a clearly distinguishable population of longer intervals.

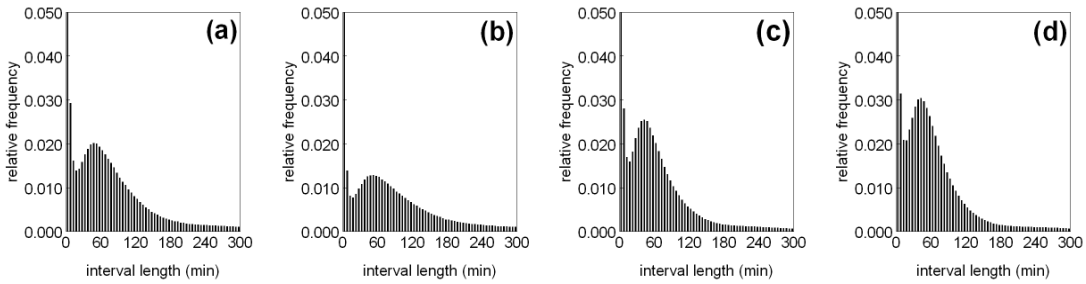

Figure 1: Histograms of the interval lengths between visits to feeders for lines A, B, C and D. A bin width of 5 min was used for all graphs and the scale of the Y-axis was chosen to best show the distribution of longer intervals. As a result, the proportion of intervals in the first bin (0.52, 0.63, 0.53 and 0.46 for lines A, B, C and D respectively) are not shown.

The degree to which feeding behaviour was organised into bouts was estimated on the basis of graphs like those in Figure 1, but determined per hatch. The ratio between the relative frequency at peak of the long intervals and that at the meal criterion (see below) did differ significantly across the lines ($F_{3,44} = 2.87$, $p = 0.047$), with line A at 1.53 ± 0.06, line B at 1.76 ± 0.09 , line C at 1.64 ± 0.04 and line D at 1.56 ± 0.05, as a result of the slightly higher value for line B.

Figure 2 shows the probability of birds starting to feed in the next minute in relation to time since the last visit. In all lines, Pstart shows a steady increase with increasing time since last visit from around 15 to 20 min. The pattern differs between the lines, with lines C and D having higher Pstart at all times than either line A or B ($p < 0.001$). When the change in Pstart was analysed, over the scale of time since the last feed shown in Figure 2, a significant difference was found between the lines ($p = 0.02$), with the ratios in change in Pstart being 1.25 ± 0.07 for line A, 1.52 ± 0.15 for line B, 1.73 ± 0.09 for line C and 1.61 ± 0.09 for line D.

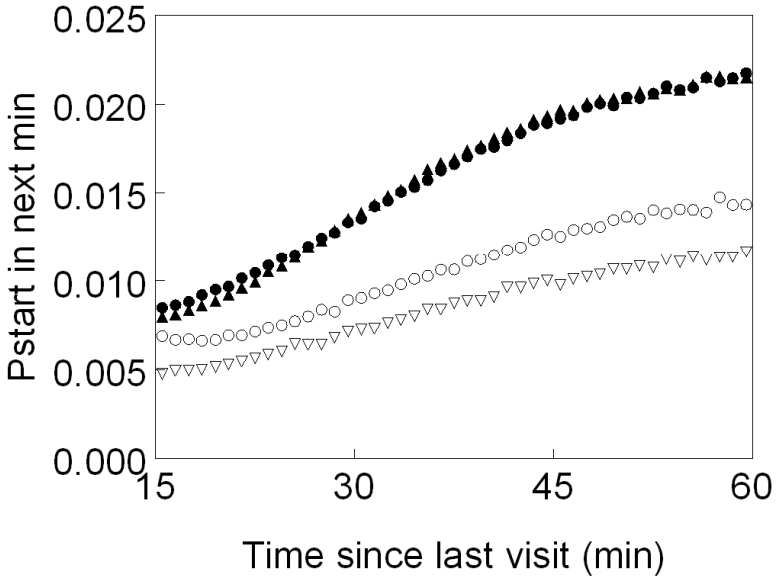

Figure 2: Probability of birds starting to feed in the next minute (Pstart) against time since last visit for line A (○), line B (▽), line C (▲) and line D (●).

3.4.3 Meal Characteristics

Table 2 shows the mean and within-line variation of the estimated meal criteria and the estimated meal characteristics for the lines. The meal criteria did not differ significantly between the lines, however all differences in meal characteristics between the lines were found to be significant at the $p<0.001$ level. Feeding rate and average daily intake were the only

characteristics to vary systematically between the lines, with line A having the fastest feeding rate and highest average intake. Number of meals per day, meal size and duration were very similar in lines A and B and lines C and D respectively, with the two faster growing lines (A & B) having fewer but larger and longer meals than the slower growing lines (C & D). There were no systematic differences between faster and slower growing lines with regards to pause time within meals or number of visits per meal.

Table 2: Mean (± standard error) meal characteristics estimated for each of the 4 lines. All characteristics were calculated using mean values per bird for the entire duration of the experiment with n = number of birds, and all with degrees of freedom = 3, 16279, except meal criterion where degrees of freedom = 3, 44

	Line A (n = 3470)	**Line B (n = 4257)**	**Line C (n = 4153)**	**Line D (n = 4403)**	**ANOVA**
Meal criterion (s)	1200 ± 40.4	1050 ± 54.1	1050 ± 35.9	1210 ± 78.8	F = 0.82
Meal size (g)	12.2 ± 0.05	13.3 ± 0.05	7.83 ± 0.03	7.27 ± 0.03	F = 5407.6 ***
Meals per day	12.2 ± 0.05	9.83 ± 0.04	15.3 ± 0.05	14.7 ± 0.05	F = 2808.6 ***
Meal duration (min)	7.38 ± 0.05	7.20 ± 0.05	5.68 ± 0.03	6.18 ± 0.05	F = 303.4 ***
Pause within meal (min)	2.17 ± 0.02	1.31 ± 0.02	1.95 ± 0.01	2.37 ± 0.02	F = 688.9 ***
Feeding rate (g/min)	2.55 ± 0.01	2.50 ± 0.01	2.35 ± 0.01	2.28 ± 0.01	F = 105.2 ***
Visits per meal	2.43 ± 0.02	3.00 ± 0.02	2.49 ± 0.01	2.16 ± 0.01	F = 509.0 ***
Average daily intake (g)	140 ± 0.26	124 ± 0.23	115 ± 0.17	101 ± 0.19	F = 5522.9 ***

3.4.4 Effect of number and weight of birds per m^2

Figure 3 shows the effect of number of birds per m^2 on meal characteristics for the 12 hatches of each line. Meal size (Figure 3a) tends to increase with bird number but some differences in meal size remain between the lines. These differences are mainly between slower and faster growing lines, with very little difference evident between lines A and B or between lines C and D. There was little systematic effect of number of birds per pen on meal duration to explain differences between lines (Figure 3b). The number of meals per day tended to decrease with number of birds per pen (Figure 3c) but the differences between lines A and B on the one hand and lines C and D on the other clearly remain. Feeding rate is not strongly affected by number of birds per pen (Figure 3d). Although the co-variable 'number of birds per pen' explained a significant part of the variation in all these characteristics ($p < 0.001$), line effects remained highly statistically significant in all cases ($p < 0.001$).

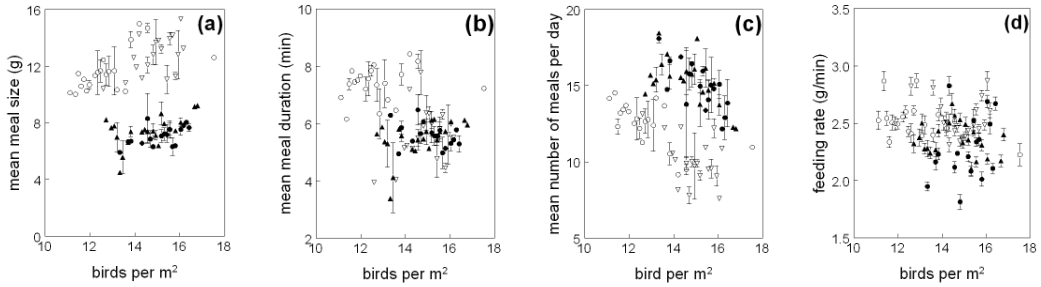

Figure 3: Mean (± standard error) of (a) meal size, (b) meal duration, (c) number of meals per day and (d) feeding rate against number of birds per m^2 for birds of line A (○), line B (□), line C (▲) and line D (●).

Figure 4 shows that differences between the lines in meal size, duration, number and feeding rate are partly explained by the effect of the average weight of birds per m^2. However, analysis showed that the differences between lines for all characteristics were still statistically significant ($p < 0.001$) when the effect of this co-variable was taken into account in the model.

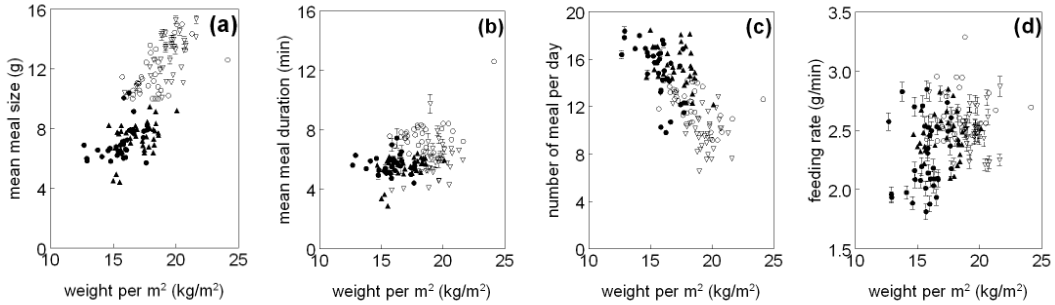

Figure 4: Mean (± standard error) of (a) meal size, (b) meal duration, (c) number of meals per day and (d) feeding rate against weight of birds per m^2 for birds of line A (○), line B (□), line C (▲) and line D (●).

The mean weight per m^2 across the lines was calculated at 17.6 kg and an estimate for each characteristic at this density was derived using regression analysis. Table 3 illustrates the predicted meal characteristics for each line if stocked at this density. As can be seen from the table, there are still large differences in meal characteristics between the lines even when birds are stocked at the same density. At the same stocking density, predicted meal duration varies systematically with growth rate, whereas predicted feeding rate does not.

Table 3: Predicted meal characteristics for average weight per pen of 17.6 kg/m^2 for each of the four lines as calculated using regression line fits on the data. REML analysis was performed on the entire data set across all weights, rather than on the predictions, to determine the effect of line when weight per m^2 and line were treated as fixed effects. The test statistic given refers to the line component of the analysis.

Characteristic	Line A	Line B	Line C	Line D	REML analysis (df = 3)
Meal size (g)	11.87	12.19	7.94	7.85	Wald stat: 4525.8 ***
Meals per day	12.64	10.50	15.05	13.16	Wald stat: 1541.2 ***
Meal duration (min)	7.46	6.86	5.70	5.54	Wald stat: 912.8 ***
Pause duration (min)	2.31	1.57	1.93	1.43	Wald stat: 319.9 ***
Feeding rate (g/min)	2.54	2.57	2.34	2.33	Wald stat: 86.7 ***
Visits per meal	2.47	3.07	2.54	1.90	Wald stat: 995.9 ***

3.5 Discussion

This study required characterisation of variables such as the frequency distribution of interval lengths between visits and the probability of birds starting to feed in relation to time since feeding last per genetic line. Accurate estimation of such variables requires large numbers of observations and for that reason characteristics were analysed with considerable number of birds per line. The smooth patterns observed in Figures 1 and 2 show that these numbers were large enough to produce very consistent patterns, suitable for further analyses.

The aim of this study was to test whether female birds intensively selected for growth showed any alteration in the structure of their feeding behaviour, which would indicate a change in the underlying intake control mechanisms. There are four scenarios of changes in STFB that could occur as a result of the increase in feed intake associated with selection for faster growth rates. In the most extreme case, there could be a complete loss of structure of feeding behaviour (i.e. no bouting at all) as a result of birds being continuously hungry, as has been suggested for birds intensively selected for growth (Burkhart et al., 1983; Bokkers et al., 2004). In this case (Scenario 1), satiation would never be achieved and the low probability of initiating feeding that is associated with satiety would not occur. Visits would then be expected to occur at random with a constant starting probability. This would, by definition, result in a single negative exponential distribution of interval lengths between visits, i.e. a continuously declining frequency distribution with increasing interval length. In Scenario 2, bouting would still occur but without effects of satiety on Pstart between (or within) meals. This is exactly the null hypothesis of much previous work on STFB and in that case the frequency distribution of intervals between visits would consist of two overlapping negative exponentials (Slater and Lester 1982; Sibly et al., 1990; Langton et al., 1995). Also, in this scenario, the frequency

distribution of interval lengths must continue to decline with each increase in interval length (Tolkamp et al., 1998). Figure 1 shows that these two scenarios clearly cannot apply to any line because the frequency distribution first declines with increasing interval length but then increases again in all graphs. In addition, the increase in Pstart for all lines shown in Figure 3 is not consistent with the underlying assumptions of either of these two scenarios. Instead, Figure 3 shows that Pstart does increase with time since the last visit at longer interval lengths, as expected when hunger and satiety control meal initiation and termination, respectively.

Alterations to the satiety and hunger mechanisms could, however, have led to a less clear distinction between the distributions of within- and between-meal intervals in association with a less pronounced effect of time since the last meal on Pstart (Scenario 3). Meal criteria estimation techniques using mixed distribution models (e.g. Yeates et al., 2001) would then not provide a good model for the data and lead to a considerable uncertainty in the estimate value, as there would be a large number of intervals that could be classed as either within- or between-meals. The data show that this was not the case as meal criteria were defined clearly for all four lines. There was, however, a statistically significant difference in bouting between the lines; this was mainly due to the high ratio of line B, as the difference is no longer significant if this line is excluded. Line B had a much higher number of records in the first bin, indicating a higher proportion of very short intervals between visits.

Models developed for estimation of meal criteria using data from these broiler birds (Howie et al., 2009) are applicable across all the lines as all show an easily distinguishable distribution of longer intervals. Overall, there is no evidence for any alteration in the pattern of STFB in any of the lines. All lines show a normal, bouted structure of feeding behaviour, i.e. clustering of

feeding bouts into meals, a clear separation of within- and between-meal interval distributions and a considerable increase in Pstart with time since the last meal (Yeates et al., 2001). This indicates that the intensive selection pressure has not changed the fundamental hunger and satiety mechanisms underlying the control of feeding behaviour in these birds, giving similar patterns to those found in both poultry and non-poultry species (Forbes 1995; Yeates et al., 2001; Bley and Bessei, 2008). This resulted in meal criteria estimates that were also not affected by genetic line (Table 2), although they were considerably longer than most of those used previously for poultry (e.g. 2 min - Masic et al., 1974; Squibb and Collier 1979; Savory 1989). These previous estimates, however, are either entirely arbitrary or are based on methods for meal criteria estimation that do not incorporate effects of satiety (Clifton 1979) and are, therefore, inappropriate (Tolkamp et al., 1998; Yeates et al., 2001).

Even though the lines show no essential alteration in the structure of STFB, the organisation of feeding behaviour into meals differs between the lines. As expected, there are systematic differences between the lines in terms of FCR, weight change and average daily intake across the experimental period, with the fastest growing line A having highest daily intake, and lowest FCR (Tables 1 and 2). Of the meal characteristics, the two faster growing lines had, on average, larger and longer meals, and slightly fewer meals per day when compared to the two slower growing lines. The most pronounced difference, after the number of birds and weight per m^2 were accounted for, was that of meal size (Figure 4a) with the slower growing birds having significantly more, but smaller, meals than birds from the other two lines. The other meal characteristics also vary between lines and there were still significant differences between each of the lines even when numbers and weights of birds were considered (Table 3).

Differences in meal characteristics between breeds and lines have been previously reported in poultry as well as other livestock species. In chickens, Masic et al (1974), using an arbitrary meal criterion of 2 min, found that heavier broiler birds ate a greater number of larger but shorter meals than layer birds. Similarly, Barbato et al (1980) found that high-weight chicks had more visits to feeders than low-weight birds, but there was no difference in intake per visit between the two lines. Labroue et al (1997) found in pigs that animals with higher daily intake ate larger, longer meals, but fewer per day, which is similar to the findings in this study with birds. However their subsequent study (Labroue et al., 1999) found that the slower growing Pietrain had fewer and smaller visits to the feeders than the larger Large Whites. Within other livestock species, there is also variation in findings of studies, with some concluding that meal size, meal frequency, both or neither differ between different breeds (Slater 1974; Davies 1977; Adenuga et al., 1991; Robinson and Oddy 2004; Bley and Bessei 2008). The variety of methods used to define meals and the resulting range of conclusions drawn from these studies (Zorrilla et al., 2005) highlight the need for a consistent method for STFB analysis to make results comparable both between studies and between species (Geary 2005).

This study has identified significant differences in STFB both within and between meals with respect to meal characteristics such as meal duration and meal size, with faster growing birds having longer and larger meals than the slower growing ones. It is possible that these differences are direct consequences of selection, although in this study there was no clear relationship between selection criteria and STFB traits. Depending on the relationship of the within-line variation of these traits with existing selection targets, such as feed conversion ratio, it may be possible to incorporate some of these traits into selection programs. The identification of the most relevant of these STFB characteristics and predicted outcomes from

their incorporation into current selection programs remains to be addressed. I conclude that although there are differences in the characteristics of feeding behaviour between the four genetic lines of broilers in this study, there is no alteration in the overall organisation of STFB into bouts and no evidence in this study that any of these intensively selected birds are constantly hungry.

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CHAPTER 4

Broilers, ducks and turkeys have a similar organisation of their short-term feeding behaviour

Revised for publication in British Poultry Science

Chapter 4 – Across species comparison of feeding behaviour

4.1 Abstract

This study tested the hypothesis that the structure of short-term feeding behaviour would be similar in broilers, ducks and turkeys that the same models would be suitable to group feeding behaviour of these species into meals. The lengths of longer day-time intervals between visits to feeders obtained with 3,470 broilers, 3,314 turkeys and 480 ducks were all log-normally distributed. Disaggregation of these intervals by feeding strategy (i.e. meal frequency) showed that the probability of birds starting to feed increased with time since feeding last in all species, which is consistent with the satiety concept. The frequency distributions of the length of short between-visit intervals varied more between species as a result of differences in the number of visits per meal, the frequency of re-visits to the same feeder and probably in the likelihood of birds drinking within meals. Two methods, one based on fitting a truncated log-normal, the other on observed changes in the probability of birds starting to feed with time since feeding last, gave very similar meal criteria estimates. These ranged from 1050 to 1200 s in broilers, 1650 to 1725 s in ducks and 1250 to 1320 s in turkeys. There were large between-species differences in the average number of daily meals, intake per meal, and feeding rate. Despite this variation, the overall structure of feeding behaviour of broilers, ducks and turkeys was so similar that the same models were suitable for analysis of short-term feeding behaviour in all three species.

4.2 Introduction

Analysis of feeding behaviour can not only improve our understanding of the mechanisms that underlie feed intake regulation (e.g. LeMagnen 1985; Zorrilla et al., 2005) but could also assist in identifying desirable traits for animal breeding programmes (Howie et al., 2009b). Because of the importance of feed consumption in animal production systems, records of visits to feeders are increasingly being collected to measure daily intake in animals such as cattle (Tolkamp et al., 1998b), pigs (Morgan et al., 2000a) and poultry (Howie et al., 2009a). Such data sets have also formed the basis for analyses of short-term feeding behaviour in each of these species (e.g. Friggens et al., 1998; Hall et al., 1999; Bley and Bessei 2008). It has been shown, however, that even small changes in feeder construction or software settings can have large effects on daily number of visits, median visit duration and intake per visit, without changing daily intake (Tolkamp et al., 2000). It is, therefore, difficult to quantitatively compare analyses of short-term feeding behaviour between studies, even if the basic unit is measured in terms of visits to feeders in the same species. This is further complicated if data are collected with species-specific feeders or by other techniques, such as direct observation (e.g. Bokkers and Koene 2003) or recording pecks (e.g. Machlis 1977), that result in different definitions of the basic unit in which feeding is expressed.

In many species, feeding behaviour is organised in bouts and these bouts, or meals, are therefore considered a more biologically relevant unit for analyses of short-term feeding behaviour (LeMagnen 1985; Forbes 1995). The grouping of short feeding events, such as visits to feeders or pecks, into meals would allow a comparison of feeding behaviour, even when data are obtained with different techniques or different species. To do this, a meal criterion needs to be estimated (e.g. Slater and Lester 1982; Langton et al., 1995). Because results of

meal pattern analysis can be greatly affected by the value of the meal criterion estimate (Zorrilla et al., 2005), it is important to demonstrate the biological basis of the meal definition and its suitability for the relevant species.

Howie et al (2009b) recently concluded that there was sufficient similarity in the structure of short-term feeding behaviour of broiler chickens of different genetic lines to allow robust estimations of reliable meal criteria by previously developed methods (Howie et al., 2009a). The study also suggested that the underlying controls of feed intake were unaffected by intensive selection for performance traits. For the current study I hypothesised that the structure of short-term feeding behaviour of Pekin ducks and turkeys would be sufficiently similar to that of broilers so that the same methodologies for the estimation of meal criteria could be used. I tested this hypothesis with data that were collected in several separate experiments, with birds of different ages and under different conditions for the three species. In addition, I tested whether or not the main principle upon which the methodologies developed by Howie et al (2009a) are based, i.e. a continuous increase in the probability of birds starting to feed with time since the last meal, applies to birds of each of these species. I also investigated how appropriate disaggregation of the data can aid in fully understanding the structure of feeding behaviour. Finally, I briefly describe the observed feeding behaviour of these three species after grouping it into meals.

4.3 Materials and Methods

Records of visits to feeders of 3,470 female broiler chickens (*Gallus gallus)*, 3,314 male turkeys (*Meleagris gallopavo)* and 480 male and female Pekin ducks (*Anas platyrhynchos domestica*) were available for analysis in this study (see summary in Table 1). The methods and equipment for obtaining the basic duck and chicken data have been previously described in detail by Bley and Bessei (2008) and Howie et al (2009a), respectively, but data were analysed here using novel methods. The turkey data were collected from a set of 16 hatches. Each hatch, consisting of around 200 male birds, was housed from 18 to 22 weeks of age in a single pen. The actual number of turkeys per pen varied slightly due to differences in the number of available birds per hatch.

Pens for broilers were equipped with one and pens for ducks and turkeys with two feeding stations. Each feeding station, previously described for ducks (Bley and Bessei 2008) and broilers (Howie et al., 2009), consisted of 8 feeders and was linked to a computer to allow automatic recording of visits. The feeding stations for turkeys were scaled-up versions of the ones used to collect broiler data. Each feeder had a back bar and side plates that were adjusted as the birds grew to allow entry of only one bird at a time. Each feeder contained a feeding tray that was linked to electronic scales to record the weight of feed at the start and end of each visit. All birds were tagged with electronic wing bands and a radio frequency antenna system was used to identify individuals entering and leaving the feeders. Start and stop time, weight of feed consumed, bird ID, visit duration, date, pen and feeder number were recorded per visit and stored electronically in a database.

Birds of all species were placed in their pens at least a few days before data collection began to allow acclimatisation to the environment and feeding equipment. Birds had *ad libitum* access to a species-appropriate pelleted feed throughout the data collection period. Lighting regimes and stocking densities differed between the species, with turkeys having the most hours of darkness and ducks having the lowest stocking density (Table 1).

Table 1: Details of experimental setup for the collection of short-term feeding behaviour data of the three poultry species

	Broilers	**Turkeys**	**Ducks**
Total number of recorded visits	1,941,822	851,513	209,309
Total number of birds	3,470	3,314	480
Bird sex	♀	♂	♀ + ♂
Number of hatches	12	16	1
Bird ages (weeks)	2 to 5	18 to 22	3 to 7
Mean start weight (kg)	0.474	21.67	1.062
Mean end weight (kg)	2.275	26.34	3.033
Pen area (m^2)	8.1	127	32
Mean birds per pen	116	207	160
Birds per feeder	14.5	12.9	10
Lighting regime (h light : h dark)	20:4	14:10	17:7

4.3.1 Analysis of feeding behaviour

The data were screened to remove visits where the wing band of the bird was not recorded, which affected less than 0.1% of the total number of broiler and turkey visits. Scale errors were also removed from the calculations of feed intake, but were included for other characteristics (less than 0.5% of all visits). On rare occasions, ducks were not properly identified by the feeders, which resulted in many short 'between-feeding' intervals which were removed before analyses.

First the interval length between two consecutive visits to feeders of the same bird was calculated. The frequency distributions of these interval lengths, as well as of $\log_e$-transformed interval lengths (measured in sec), were plotted to assist in determining the structure of short-term feeding behaviour and the estimation of meal criteria. On the basis of previous experience, bin-widths of 5 min and 0.5 $\log_e$-units were selected to present the clearest graphs.

The effects of data pooling (Morgan et al., 2000b; Yeates et al., 2003) on the distribution of interval lengths and on starting probabilities were investigated by disaggregation of the data in to sub-sets. First, 'day-time' data sets were produced for each species that consisted of intervals that occurred entirely during the light period only. For each species, day-time intervals of all birds were further disaggregated into two groups, i.e. (i) intervals between visits to the same feeder and (ii) intervals between visits to different feeders. After meal criteria were estimated (see below) three additional data sets were created of birds with similar feeding strategies in each species for further analysis. To that end, first the mean number of daily meals were calculated per bird. For each species, data of three sub-groups of 50 birds each were pooled. These sub-groups consisted of birds with a mean daily meal number that was (i)

around the average or (ii) the lowest or (iii) the highest of the total population. To further investigate the structure of short-term feeding behaviour of ducks, I calculated for each duck the number of intervals with an intermediate duration (i.e. between 1 and 10 min) as a proportion of all intervals between visits to feeders. Two data sets were created by pooling the data of 50 ducks each with either (i) the lowest or (ii) the highest proportion of such intermediate intervals.

Two methods were used to estimate meal criteria per hatch. First a truncated log-normal model was fitted to the distribution of $\log_e$-transformed between-visit interval lengths using GENSTAT (VSN International 2008), as described in detail by Howie et al., (2009a). The model was fitted to the lengths of day-time intervals longer than 7.5 $\log_e$ units and has the following probability density function (PDF):

$$PDF = (1/cunormal((7.5-\mu)/\sigma))(1/(2.506628\ \sigma))exp(-((x-\mu)^2)/(2\sigma^2))$$

Where: cunormal = GENSTAT cumulative normal correction factor to allow for truncation, μ and σ = mean and standard deviation of the log-normal distribution, and x = $\log_e$ interval length (in sec). The meal criterion was estimated at the interval length where the number of observed intervals was twice that predicted by the model, as outlined by Howie et al (2009a).

Meal criteria were also estimated on the basis of the probability of birds starting to eat in relation to time since feeding last (P_{start}). The probability of birds starting to feed within the next 5 min at time t since feeding last was calculated as the number of intervals > t and ≤ t + 5 min divided by the number of intervals > t min (Tolkamp and Kyriazakis 1999a). For the

estimation of meal criteria, these probabilities were also calculated with bin-width of 1 min. As described previously (Howie et al., 2009a), the meal criterion is estimated as the time since feeding last where P_{start} is lowest, using a rolling average over 5 minute intervals to reduce the effect of random variation in values.

Meal criteria according to both methods were estimated per hatch. ANOVA was used to test for an effect of methodology of estimation on resulting meal criteria estimates and the associated meal characteristics.

4.4 Results

4.4.1 Distribution of interval lengths between visits

Figure 1 (top row) shows the relative frequency distributions of the length of intervals between visits to feeders for all data pooled per species. Broilers, turkeys and ducks all showed a rapid decrease in frequencies with increasing interval length to a minimum between 15 and 30 min. The frequencies then increased to a peak between 50 and 80 min, and then gradually decreased to very low frequencies at long intervals. Although the values of the minima and maxima varied between the species, all species show a similarly shaped distribution of between-visit interval lengths.

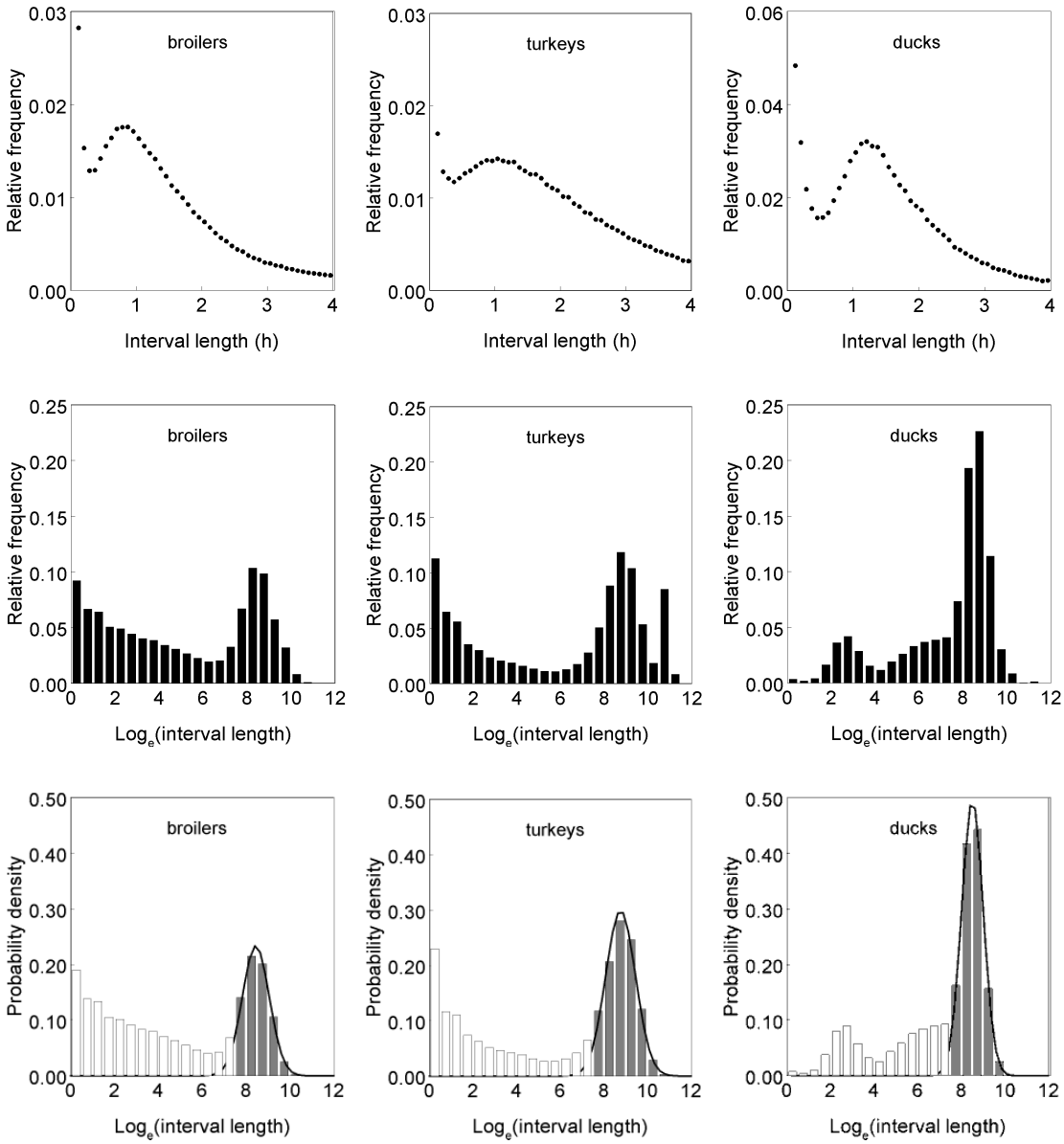

Figure 1: **Frequency distributions of (log$_e$-transformed) interval lengths between visits to feeders recorded with broilers, turkeys and ducks**. The top row graphs show plots of the frequency distributions of all interval lengths for each species, using a bin-width of 5 min; the values for the first bin (0.55, 0.41 and 0.22 for broilers, turkeys and ducks, respectively) are not shown to allow a better view of the pattern at longer interval lengths. The centre row shows histograms of all log-transformed lengths of intervals between visits to feeders; bars are the observed relative frequencies (bin-width 0.5 log$_e$-units). The bottom row shows the

frequency distribution of $\log_e$ -transformed lengths of intervals between visits after removal of intervals that include (part of) the dark period; bars are the observed frequency distribution divided by bin-width (0.5 $\log_e$-units), with solid bars for data to which the truncated probability density function (see text) was fitted, shown here in bold lines.

Log_e-transformation of interval lengths resulted in the distributions shown in Figure 1 (centre row). From the data obtained with turkeys, it was evident that there was a population of very long intervals, i.e. longer than approximately 10.5 $\log_e$-units (corresponding to 10 h), that was almost entirely separated from the remainder of the interval distribution. These very long intervals all included at least part of the dark period. When the three data sets were disaggregated by removing all intervals that included the entire dark period or parts thereof, the separate distribution for very long intervals in turkeys completely disappeared (Figure 1, bottom row). The same procedure also removed 'blips' that were previously observed in the frequency distribution of long $\log_e$-transformed intervals for chickens (around 9.6 $\log_e$-units, corresponding to 4 h) and ducks (compare centre with bottom row graphs in Figure 1). The remaining distributions of the population of longer $\log_e$-transformed intervals were approximately normal in all species. Figure 1 shows that, apart from the approximately log-normal distribution of a population of longer intervals, there is at least one additional population of shorter $\log_e$-transformed interval lengths for broilers and turkeys and there are at least two for ducks. These are investigated in more detail below.

4.4.2 Changes in probabilities of birds starting to feed

Figure 2 (top row) shows how the probability of birds starting to feed within the next five minutes changed with time since birds fed last as estimated for the data pooled per species. An initial decrease in P_{start} to a nadir between 20 and 30 min was followed by an increase to a plateau or even a later decrease. As this may be an effect of pooling across day and night data (Morgan et al., 2006b), the intervals that included (part of) the night period were removed, resulting in the probabilities depicted by the graphs in the middle row of Figure 2. The bottom row of Figure 2 shows the effects of disaggregation by feeding strategy on P_{start} estimates. In contrast with the trend seen in the pooled data, P_{start} generally continued to increase with time since feeding last for birds with a given feeding strategy.

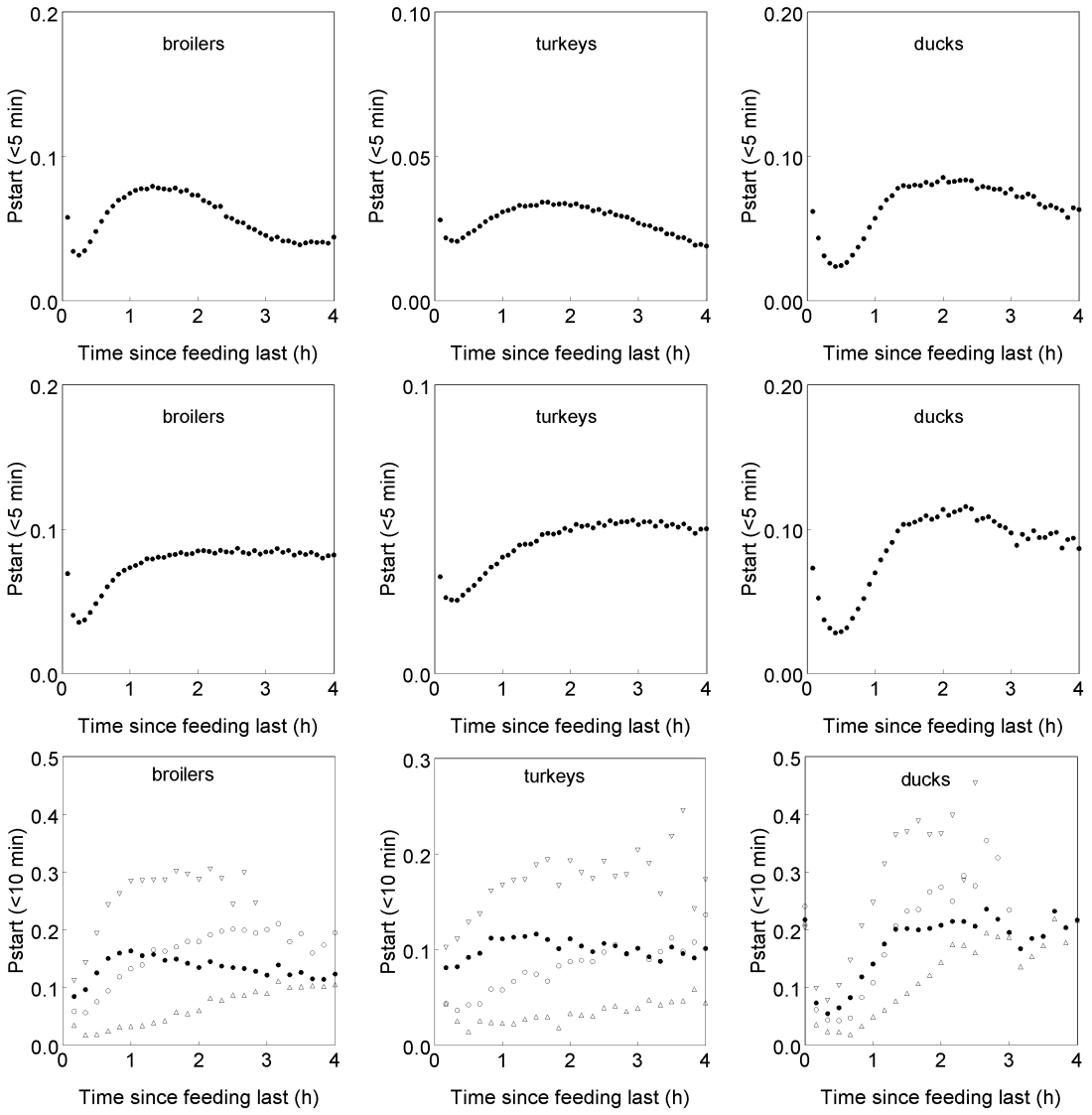

Figure 2: **Probability of birds starting to feed within the next 5 minutes (P_{start}) in relation to the time since they fed last** for the pooled data of broilers, ducks and turkeys (top row graphs). To better show the changes in P_{start} at longer intervals, the values for the first bin (i.e. the probability of birds starting to feed within 5 min of feeding last) have not been plotted for broilers and turkeys (values were 0.50 and 0.41, respectively). The centre row shows the data for the three species pooled across intervals occurring during the light period only. The graphs in the bottom row show the species-specific P_{start} (within 10 min, to accommodate the lower number of observations) that were calculated for groups of 50 birds after data were

disaggregated by feeding strategy (Δ = few daily meals, ○ = average number of daily meals, ∇ = many daily meals and ● = data combined).

4.4.3 Effect of disaggregation of interval data by feeder visited

For ducks, with few very short between-visit intervals, the sub-set of day-time intervals between visits to the same feeder had virtually the same distribution as the sub-set of day-time intervals between visits to different feeders (Figure 3). Disaggregation of data obtained with broilers and turkeys, however, showed that very short intervals were recorded between visits to the same feeder only (Figure 3, centre row graphs) and not between visits to different feeders (Figure 3, bottom row graphs). For broilers, the frequency distributions of the $\log_e$-transformed length of intervals between visits to different feeders clearly showed two overlapping populations that were both approximately log-normally distributed (Figure 3). For turkeys, the data suggest that there is a population of shorter intervals, similar to that observed in broilers but much smaller, that overlaps with the log-normally distributed population of between-meal intervals.

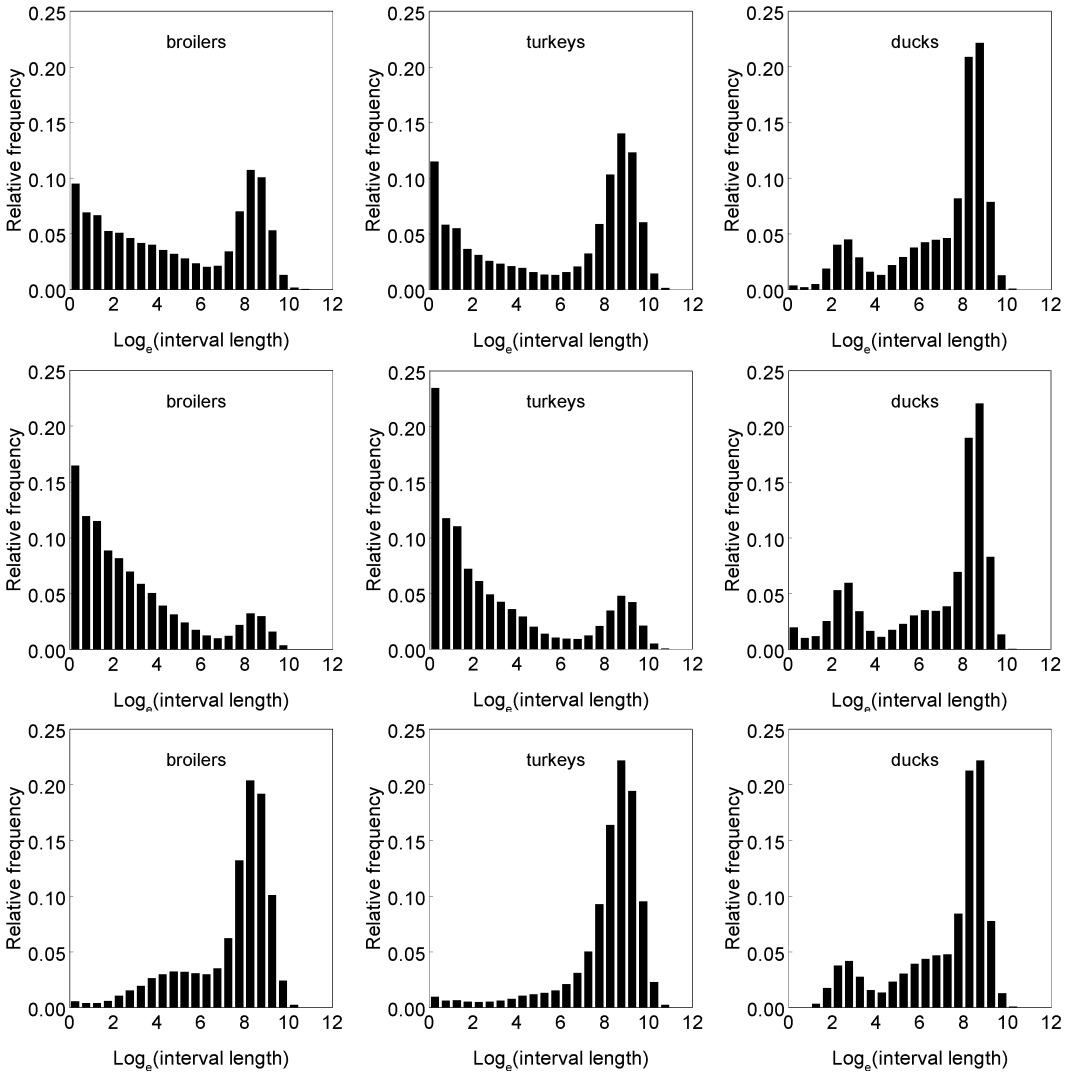

Figure 3: Frequency distributions of pooled log-transformed interval lengths between visits to feeders recorded with broilers, turkeys and ducks after deleting intervals that include (part of) the dark period (all bin widths 0.5 $\log_e$-units). The graphs show the frequency distributions of the pooled $\log_e$-transformed intervals (top row), intervals between visits to the same feeder only (centre row) and intervals between visits to different feeders only (bottom row).

4.4.4 Effects of disaggregation of duck data by individual feeding strategy

Duck data were examined in more detail in an attempt to find an explanation for the occurrence of three populations of intervals between visits to different feeders rather than the two that were observed in birds of the other two species. A preliminary inspection of interval distributions obtained with individuals showed that there was little variation between ducks in the distribution of the populations of very short (with a peak around 2.75 $\log_e$-units corresponding to 15 sec) and very long (with a peak between 8 and 9 $\log_e$-units, corresponding to 50 and 135 min) intervals. There was, however, considerable individual variation in the number of intervals between 1 and 10 min, corresponding to 4.1 and 6.4 $\log_e$-units, as a percentage of all intervals (from 1 to 46%). Figure 4 shows the observed frequency distributions of interval lengths pooled across the 10% of individuals with a low or a high proportion of intervals in the range of 1 to 10 min. The figure demonstrates that a considerable proportion of ducks showed little evidence of a third population of intervals. At the same time, another considerable proportion of ducks produced a relatively large third population with 'intermediate' lengths (i.e. from around 1 to around 10 min) that was very clearly distinguished from the other two populations of intervals (Figure 4).

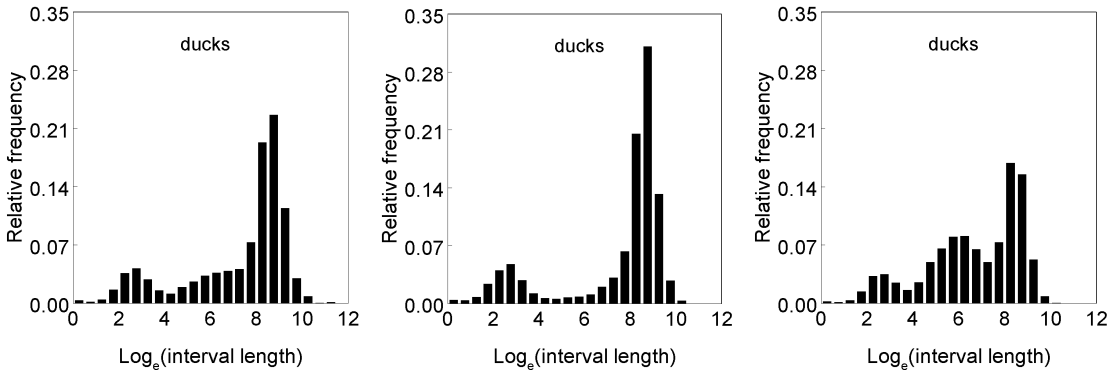

Figure 4. Frequency distributions of log_e-transformed intervals between visits to feeders that were observed in ducks (bin widths 0.5 log_e-units). Graphs show the distribution of all data pooled (left-hand graph) and of data pooled across 48 birds (10% of the total) with a number of intervals between 1 and 10 min that, as a proportion of all intervals, was either lowest (from 0.01 to 0.04; n = 12,303; centre graph) or highest (from 0.21 to 0.48; n = 22,865; right-hand graph)

4.4.5 Estimation of meal criteria

A truncated normal describing all pooled log_e-transformed day-time intervals longer than 7.5 log_e-units gave a good fit to the data pooled across species (Figure 1, bottom row graphs). The model converged easily for all data sets and species-specific meal criteria could quickly be estimated (Table 2). Similarly, a clear trough in the plot of the probability of birds starting to feed in relation to time since feeding last (Figure 2, centre row) allowed an easy estimate of meal criteria for each species by this method. . Meal criteria estimated per hatch in this manner were 150, 70 and 75 seconds shorter for broilers, ducks and turkeys, respectively, than estimates resulting from the truncated normal method. Because of the scarcity of intervals in this range, the effect of these shorter meal criteria on average number of daily meals (an

increase with less than 0.1 in each species) and other meal characteristics was, however, very small. Meal criteria estimated by the truncated log-normal method were used to group visits into meals for further analyses (i.e. all between-feeding intervals shorter than the meal criterion were ignored for the calculation of meals).

Table 2: **Comparison of meal criterion estimates for broilers, turkeys and ducks** on the basis of a truncated log-normal model or changes in the probability of birds starting to feed (Pstart) in relation to time since feeding last (see text). The effect of the different criteria on the estimated average number of daily meals is also given.

		Broilers	Turkeys	Ducks
Trunc normal	**Meal criterion (s)**	1200	1320	1725
	Meals per day	12.2	6.21	12.2
Pstart	**Meal criterion (s)**	1050	1250	1650
	Meals per day	12.3	6.27	12.3

4.4.6 Meal characteristics

Broilers and ducks had a very similar average daily number of meals but turkeys consumed their feed in considerably fewer meals (Table 3). Ducks not only had the shortest meals but also spent the smallest proportion of total meal duration feeding (24.1% vs. 70.6% and 81.8% for chickens and turkeys, respectively). This resulted in ducks having by far the shortest daily feeding times (less than 12 min/day, compared with 34.2 and 63.6 min for turkeys and broilers, respectively; Table 3).

Table 3: **Summary of mean (± standard error) meal characteristics for broilers, ducks and turkeys.** Meal criteria were estimated using the truncated log-normal model.

	Broilers	Turkeys	Ducks
Meal criterion (sec)	1200 ± 40.4	1320 ± 78.0	1725
Meals per day	12.2 ± 0.05	6.2 ± 0.04	12.2 ± 0.11
Meal duration (min)	7.38 ± 0.05	6.47 ± 0.07	4.02 ± 0.08
Meal size (g)	12.2 ± 0.05	140 ± 0.82	19.31 ± 0.20
Time spent feeding per meal (min)	5.21 ± 0.02	5.29 ± 0.02	0.97 ± 0.08
Feeding rate (g/min)	2.55 ± 0.01	30.3 ± 0.21	20.73 ± 0.21
Visits per meal	2.43 ± 0.02	1.87 ± 0.02	1.53 ± 0.01
Daily intake (g)	140 ± 0.26	804 ± 3.60	227 ± 1.43
Time spent feeding per day (min)	63.6 ± 0.13	34.2 ± 0.18	11.8 ± 0.17

4.5 Discussion

The main objective of this study was to test the hypothesis that the same methods could be applied for the estimation of meal criteria in chickens, ducks and turkeys because of a similar structure of their feeding behaviour. The distribution of longer (i.e. between-meal) intervals has a major effect on meal criteria estimates and this distribution is, therefore, discussed first.

4.5.1 The structure of feeding behaviour: the distribution of longer intervals

Plots of the frequency distribution of $\log_e$-transformed interval lengths between behavioural events can reveal much about the structure of animal behaviour (Tolkamp et al., 1998a; Yeates et al., 2001). Figure 1 shows that the distribution of interval lengths between visits was clearly not consisting of just a single interval population in any of the three species, which is evidence for a bouted structure of behaviour. .Most of the longer intervals in each species were part of a skewed normal distribution that could be normalised by $\log_e$-transformation of interval lengths. Diurnal species such as pigs (e.g. Morgan et al., 2000b), cows (e.g. Yeates et al., 2003) and chickens (Savory 1980; Howie et al., 2009a) have a much lower probability of feeding in dark than in light conditions. As a result, between-feeding intervals are on average longest when these include part or all of the dark period. In the present analysis, this was shown most clearly by the separate population of very long $\log_e$-transformed interval lengths in turkeys (Figure 1). This is likely to be related to the comparatively long dark period (10 h) that turkeys were subjected to, but the effect was also observed for ducks and broilers, exposed to dark periods of, respectively, 7 and 4 h only (Figure 1). Because such long intervals will always exceed meal criteria, I disaggregated the data set for those analyses by removing intervals that included (part of) the dark period to facilitate model fitting.

The remaining pooled longer intervals seemed to form a single, homogeneous and log-normally distributed population in ducks and turkeys (Figure 2), as was previously observed in broilers (Howie et al., 2009a; 2009b). This is further justification for the fitting of a log-normal to the pooled population of long day intervals to estimate meal criteria.

A log-normal distribution of intervals between specific behaviour can only be expected, however, if the probability of animals expressing that behaviour first increases but subsequently decreases with time since that behaviour was last expressed (Yeates et al., 2001). Such a decrease in starting probability at longer times is, however, in conflict with an expectation based on the satiety concept (Metz 1975; Simpson 1995; Yeates et al., 2001). Pooling of between-feeding intervals across day and night or across individuals with different feeding strategies can obscure real changes in starting probability with time since the last meal for an individual at a given time (Morgan et al., 2000b; Yeates et al., 2003; Howie et al., 2009a). For that reason, I investigated (Figure 2) the effects of disaggregation of the pooled data by feeding strategy on the change in P_{start} with longer times since feeding last. A bird with many daily meals has many short and very few long intervals between meals. In contrast, a bird with few daily meals will have few, mainly long, intervals between meals. Separately analysed, such data will result in a higher average P_{start} for the bird with many than for the bird with few daily meals at any time since the last meal. However, even if P_{start} increases for both birds with time since the last meal, this may not be so when the data of the birds are pooled (Morgan et al., 2000b). In the pooled data set, the combined P_{start} will be relatively high initially and will first increase with time. This is because at short times it is determined mainly by the many short intervals obtained with the bird that has many daily meals. At longer times, however, P_{start} may plateau or even decrease again as it will be increasingly affected by the

(relatively low) P_{start} associated with the bird that has few daily meals. Since young/small birds usually have more daily meals, and therefore a higher P_{start}, than older/larger birds (Howie et al., 2009a, 2009b), disaggregation by age is in essence also a disaggregation by feeding strategy. Figure 2 shows that in all species, when data were properly disaggregated, P_{start} continued to increase with time since the last meal, in a manner that can be expected on the basis of the satiety concept (Yeats et al., 2001). In the pooled data set, however, the estimated P_{start} is consistent with a log-normal distribution of pooled intervals between meals in broilers, turkeys and ducks and this resulted in similar distributions of longer between-feeding interval lengths in all three species.

4.5.2 Estimating meal criteria

Figure 1 shows that there were large numbers of short intervals, especially for broilers and turkeys, that were clearly not part of the log-normal distribution of the population of longer, i.e. between-meal, intervals. It was not immediately clear how many populations of short intervals there were and how each of these was distributed. For such situations, Howie et al (2009a) developed two methods that allowed estimation of meal criteria on the basis of broiler data. The method based on fitting a truncated $\log_e$-normal model to the distribution of long intervals only, suited the duck and turkey data as well (Figure 1, bottom row). The second method developed with broilers relies on estimation of the trough in starting probabilities calculated from pooled data (see Howie et al., 2009a for justifications of each of these methods). The present analyses showed that such a trough is also clearly observed in data obtained with ducks and turkeys (Figure 2). Meal criteria that were estimated with this, rather than the truncated log-normal method, resulted in similar predictions of daily meal number for all species (Table

2). That shows that both methods developed for broilers can be successfully applied to estimate meal criteria in turkeys and ducks.

4.5.3 The structure of feeding behaviour: the distribution of shorter intervals

In previous analyses of broiler feeding behaviour, it was not well understood what caused the observed distribution(s) of intervals shorter than the estimated meal criterion (Howie et al., 2009a; 2009b). The hypothesis that disaggregation of data in subsets of intervals in which drinking did or did not occur would assist model fitting was tested for broilers but proved to be wrong (Howie et al., 2009a). In the present study I further explored the use of disaggregation of pooled data into subsets to elucidate the origin of these short between-feeding intervals. From this analysis it became clear that very short intervals were not recorded between visits to different feeders. It will take more than a few seconds for a bird to leave a feeder, move to another one (certainly if adjacent feeders are occupied by other birds) and access that feeder. This explains the absence of very short intervals between visits to different feeders. It is not immediately clear, however, what caused the considerable number of very short intervals between visits to the same feeder in broilers and turkeys. Around 16% and 22% of all intervals between visits to the same feeder had a duration of a single second and a further 32% and 30% lasted from 2 to 7 seconds in broilers and turkeys, respectively (Figure 3). It seems unlikely that broilers or turkeys could actually completely leave a feeder and access it again in a period as short as one (or even a few) sec. It seems more likely that a temporary loss of contact between the tag on the bird and the RFI antenna system could occur for certain positions of a bird remaining in a feeder. This may have resulted in the high frequency of very short 'intervals' between visits recorded for broilers and turkeys.

In the duck data, such very short intervals occurred only sporadically. This could well have been caused by a difference between feeder systems in the sensitivity of continuous bird identification. In the pooled duck data, log-transformation of interval length suggested that there were three populations of between-feeding intervals (Figure 1). Inspection of individual data showed that a log-normally distributed population of short intervals and a log-normal distribution of long intervals (with a peak between 1 and 2.5 h) occurred in all birds. A proportion of birds, however, showed another clear population (consisting of up to almost half of all intervals) with an intermediate length between approximately 1 and 10 min that was virtually non-existent in other birds (Figure 4). This suggested clear individual differences between ducks in the structure of short-term feeding behaviour. A similar individual variation in the number of populations of between-feeding intervals has been observed in cows (Tolkamp and Kyriazakis 1999). Some cows, but not others, were observed to visit the water trough and drink during within-meal intervals and this, obviously, increased the lengths of these intervals compared to intervals during which no drinking occurred. This resulted in cows that showed clearly three or only two populations of intervals between visits to feeders that were (approximately) log-normally distributed (Tolkamp and Kyriazakis 1999; Yeates et al., 2001). Similarly, Zorrilla et al (2005) concluded that in rats meal criteria can only be properly estimated if the occurrence of within-meal drinking pauses is taken into account. Unfortunately, no observations on drinking behaviour were made during data collection with ducks. It is tempting, however, to think that differences between ducks in drinking behaviour could be behind the individual differences in the distribution of interval lengths. There may be a number of reasons why within-meal drinking would occur in ducks while there is no evidence that it occurs frequently in broilers (Howie et al., 2009) or turkeys. Ecological differences in the original habitats of the involved species would suggest that dry feed (as fed

during these experiments) may have been less common in the feeding environment of ducks compared to birds of the other species. The collected data also show a considerable difference between species in feeding rate. Whereas the age and body weight of the ducks overlapped with that of broilers and duck age and weight was certainly lower than that of turkeys (Table 1), ducks showed by far the highest feeding rate. This may well be a direct result of the differences between species in bill-shape, with duck bills especially suited to scoop up large quantities of feed pellets in a short period of time. Perhaps birds that consume such large amounts of dry feed per minute are more likely to become thirsty during a meal than birds of species with a lower feeding rate. Ducks not only had the highest feeding rate, they were also estimated to spend the lowest proportion of total meal time feeding (less than a quarter compared with more than 70% in broilers and turkeys). This would be consistent with part of the ducks using some of the within meal intervals for drinking, which would result in longer within-meal intervals that can be seen as a separate population in at least a proportion of individuals. Until data on drinking behaviour in relation to feeding in ducks are available for analysis, however, the suggestion developed here must remain tentative.

4.5.4 Meal patterns

On average, turkeys consumed their daily intake in only half the number of meals compared with chickens and ducks and many of these meals consisted of a single visit only. However, this may not be solely a species effect. The available turkey data were from birds that were older and at a higher proportion of their mature size than the birds from the other two species. Howie et al (2009a; 2009b) observed a trend of fewer and larger meals in several broiler lines as birds increased in age and weight and this will have affected any comparisons of meal

patterns across species here. In addition, birds of all species consumed most of their feed during the light period. The light period was shortest in the experiment collecting turkey data. This will have stimulated turkeys to consume their daily intake in a shorter time period. This will have resulted in a relatively higher bird pressure per feeder in turkeys compared with other species than is suggested by the data in Table 1. Such an increase in animal pressure per feeder may well have contributed to increased visit and meal sizes, as has been observed in other species (Nielsen et al., 1995).

4.6 Conclusions

I conclude that the frequency distribution of longer, i.e. between-meal, intervals was very similar in broilers, ducks and turkeys. This was a direct result of similar changes in the probability of birds starting to feed with time since feeding last. As a result, methods developed by Howie et al (2009a) for the estimation of meal criteria in broilers, based on fitting a truncated log-normal or on analysis of changes in P_{start} could be applied to data obtained with turkeys and ducks as well. This was despite considerable differences between species in the distribution of shorter, i.e. within-meal, intervals. Part of these differences, especially the frequency of extremely short intervals between visits, seems to be related mainly to differences in the efficiency of ID detection of birds remaining in the same feeder. Another part is possibly related to differences between part of the ducks on the one hand and broilers, turkeys and the remainder of the ducks on the other in the occurrence of drinking during within-meal intervals. Disaggregation of data that were originally pooled across day and night, across all individuals and across visited feeder numbers provided a powerful tool to further the understanding of the structure of short-term feeding behaviour in the poultry species considered here. The analysis of such behaviour will not only improve our understanding of the role of hunger and satiety in

feed intake regulation (Howie et al., 2009b) but could also provide additional traits for selecting the most desirable future genotypes of domestic poultry species.

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CHAPTER 5

Genetic parameters of feeding behaviour traits and their relationship with live performance traits in modern broiler lines

Chapter 5 – Heritabilities and genetic correlations

5.1 Abstract

Current selection goals in broiler chickens focus on the improvement of live performance traits, such as FCR. As such traits involve daily feed intake, it is possible that selection for them may have also affected their underlying traits, such as feeding behaviour. Feed intake can be split into component feeding behaviour traits, allowing the estimation of genetic correlations with FCR and assessment of the genetic consequences of selecting for FCR on feeding behaviour traits. The development of electronic feeders allows measurements of individual feed intake of birds housed in large groups, as well as the identification of feeding behaviour traits for genetic analysis. To investigate the genetic relationships between FCR and feeding behaviour, data of visits to feeders by birds from four lines of broilers, differing in their selection focus for growth and FCR, were analysed. Visits were recorded electronically and grouped into meals using existing models for estimating meal criteria. Mean feeding behaviour traits were then calculated per bird across the entire test period (2 to 5 weeks old). Feeding behaviour traits analysed were: meals per day, intake per meal, visits per meal, meal duration, non-feeding time in meal, time feeding per day, proportion of meal spent feeding, feeding rate and daily feed intake. The components of FCR analysed were body weight at 35 days and "lifetime" feed intake (14 to 35 days). All feeding behaviour traits showed moderate to high heritabilities (0.24 to 0.57), but low correlations with performance traits (-0.20 to +0.18), except for daily intake which showed moderate correlations with lifetime feed intake (+0.57). The low correlations indicate that previous selection has not significantly affected feeding behaviour traits. All lines showed similar genetic correlations and heritabilities, suggesting feeding behaviour traits are highly conserved across lines. Different feeding strategies to achieve the same FCR goals were identified within lines and this, together with the low correlation with FCR traits, would allow continued selection for FCR with little impact

on the intrinsic feeding behaviour. It would also allow, if desired, the potential for selection for different feeding strategies within lines without compromising continuous improvements in FCR.

5.2 Introduction

Feed costs have a large effect on the profitability of poultry production systems, accounting for up to 70% of total production costs (Waller 2007). Consequently, selection targets focus on improving the feed conversion ratio (FCR) of animals whilst maintaining or improving other traits, such as body weight, breast yield, leg and metabolic health and liveability (Laughlin 2007). Improvements in FCR have been so far achieved without direct consideration of traits related to feeding behaviour, as FCR only considers the feed intake over a period of time, rather than the underlying feeding behaviours. However, it is possible that focus on feed conversion traits may affect underlying traits, such as feeding behaviour, which is the constituent component of feed intake. It has been suggested that selection for performance traits in broiler chickens has adversely affected the underlying controls of feed intake with the possibility that these birds are continuously hungry (Bokkers et al., 2003, Burkhart et al., 1998). If this is the case it would have major welfare implications.

Individual daily feed intake of group-housed animals can be measured accurately with electronic feeding systems, which typically record behaviour in terms of visits to feeders (e.g. Bley and Bessei 2008). However, analysis of the short term feeding behaviour of animals on the basis of visit to feeders may lead to inappropriate conclusions (Tolkamp and Kyriazakis, 1998). With the recent development of novel models to estimate meal criteria (Yeates et al., 2001; Howie et al., 2009a,b), these visits can now easily be grouped into meals, which are more biologically relevant units of feeding behaviour than visits (Tolkamp et al., 1998, 2000). This means that reliably determined meal-based feeding behaviour traits of broilers are now available for genetic analysis for the first time. Estimation of the genetic correlations between traits allows assessment of how selection for one trait impacts on these other traits, and thus the

possible detrimental effects of continued selection on traits which are not currently considered in breeding programmes.

Individual animals can exhibit different feeding strategies to achieve the same feed intake (e.g. Tolkamp et al., 1998). This variation between individuals could be used in a selection program to produce strains of birds that all use a similar feeding strategy, which may be more suitable for a particular environment. Selection for a particular feeding strategy may aid in enhancing animal welfare in less optimal conditions if, for example, animals that take a smaller number of meals or eat at a faster rate are preferred when stocking density conditions are high (e.g. Nielsen et al., 1995).

The aims of the study were: (i) to determine genetic parameters for a range of meal-based feeding behaviour traits, and their genetic relationship with the components of feed efficiency; (ii) to establish whether past and ongoing selection has had any effects on feeding behaviour at a genetic level and (iii) to identify feeding behaviour patterns and their potential for use as future selection candidates. To do this, data from four lines of broiler chicken, differing in their focus of selection for growth were used and genetic parameters were estimated per line for a range of feeding behaviour and performance traits.

5.3 Methods

5.3.1 Animals and Housing

The experimental details of this study were similar to those described by Howie et al (2009b). In brief, data from visits to feeders by female birds from four lines of broilers were available for analysis. The lines mainly differed in intensity of selection for growth, with lines A to D having the highest to lowest growth, attaining weights of 2.4 kg, 2.1 kg, 1.9 kg and 1.6 kg by 35 days of age, respectively. The selection emphasis was also different between the lines, with the two faster growing lines focusing on body weight and FCR and the two slower growth lines on body weight, fertility and egg production. Birds were housed in the experimental pens from hatching to allow adaptation to the feeding system and environment but the data collection period lasted 21 days between 2 to 5 weeks of age. Each pen measured 8.1 m^2 and contained 8 feeders, with an average feeder pressure across the lines of 14.5 birds per feeder. This feeder pressure was not expected to affect the feeding behaviour of the birds through competition. Three pens of birds per line were available for each hatch, and all lines in a hatch were housed together in the same sheds. Numbers of birds varied between lines, due to differences in the number of chicks available per hatch. Feeding behaviour data were available for 14,048 birds for line A; 18,092 for line B; 14,044 for line C and 14,048 for line D, which covered a period of approximately a year per line (~52 hatches). Body weight data were collected separately and were available for 2 years of birds per line.

5.3.2 Feeders and Behaviour Recording

All birds had *ad libitum* access to a high quality pelleted diet, containing 210 g crude protein and 13.3 MJ metabolisable energy per kg feed, throughout the test period. The feeders allowed access of one bird at a time by means of adaptable side plates and a back bar, which were

adjusted as the birds grew. Each bird was identified by an electronic wing band, which could be read by the sensors as a bird entered and left the feeder using a radio frequency antenna system. The start and stop time of each visit to a feeder was recorded, along with the weight of feed consumed and ID codes for the bird, feeder, pen and shed. The visits were screened to remove those in which the wing band was not recorded correctly (<0.1% of all visits). Visits in which less than -0.1 g of feed was recorded as consumed (< 0.5% of all visits) were removed from intake calculations, but were retained for calculating the other feeding behaviour traits.

5.3.3 Feeding Behaviour Trait Calculations

Visits to the feeders of each individual bird were grouped into meals after estimation of a suitable meal criterion per line, using the method developed by Howie et al (2009a). Firstly, a truncated log-normal model was fitted per hatch for 12 hatches, one per month, to the distribution of log-transformed intervals between visits for intervals longer than 1800 s (7.5 $\log_e$ units), using a maximum log-likelihood approach in GENSTAT (VSN International, 2008). A meal criterion was then estimated at the point at which the model accounted for exactly half of the observed frequency of intervals and a mean meal criterion was then calculated per line as the average of these hatch criteria as discussed by Howie et al (2009b). Meal criteria estimates for lines A to D were 1200 s, 1050 s, 1050 s and 1210 s respectively. Previous analysis showed that the differences between these estimates had no biologically significant effect on the values of feeding behaviour traits obtained (Howie et al., 2009b). Consecutive visits by the same bird that were separated by intervals shorter than the meal criterion were grouped together into meals and mean meal traits were calculated per bird over the entire duration of the test period. Nine feeding behaviour traits were analysed: number of meals per day, meal duration (s), intake per meal (g), non-feeding time in meal (s), feeding rate

(g/min), daily intake (g), number of visits per meal, time spent feeding per day (min) and proportion of the meal spent feeding.

5.3.4 Genetic Analysis

Heritabilities (h^2) and genetic correlations of feeding behaviour traits and the components of FCR, bodyweight at 35 days (BWT) and "lifetime" feed intake between 14 and 35 days, adjusted for body weight (LFI), were estimated per line by REML analysis using PEST and VCE programs (Groeneveld et al., 1992). Multi-trait variance component analysis was estimated using animal models including the fixed effects of hatch, pen, mating group and sex, and random effects of the common environmental effects (c^2) and animal genetic effect. Data were available for two and a half generations (i.e. two years) of birds per line, including between 100,000 and 160,000 birds per line. Estimated breeding values (EBVs) were calculated for meal size, body weight and lifetime feed intake by PEST (Groeneveld et al., 1992). EBVs were expressed as within-line mean deviations.

5.3.5 Characterizing Feeding Strategies

To determine whether it would be possible to select birds with the same body weight/lifetime feed intake but different feeding strategies, both phenotypic records of STFB traits and estimated breeding values (EBVs) were used. Firstly, using line A as an example set of birds, phenotypic records of traits were estimated per bird and adjusted for the fixed effects of hatch and mating group. A subset of birds with the best FCR (top 25%) was analysed to estimate which feeding strategies were associated with the best feeding efficiency. Phenotypic values for body weight and lifetime feed intake were then plotted against meal size for these birds.

To establish the potential effects of selection on feeding behaviour, estimates of EBVs for meal size for a single hatch of birds in line A were compared graphically to determine whether it would be possible to select for birds with the same body weight/lifetime feed intake but different meal sizes. As a reference point, the mean body weight and lifetime feed intake was used to compare the range of mean meal sizes currently seen in birds of the same weight/mean feed intake.

5.4 Results

5.4.1 Feeding Behaviour Traits

Table 1 shows the means per line for the feeding behaviour traits. Clear differences were shown in the feeding behaviour traits between the lines, with the faster growing lines A and B having longer, larger but fewer meals than the two slower growing lines. Feeding rate, average daily intake and time spent feeding per day and per meal were also higher in the faster growing lines.

Table 1: Means (± standard errors) of feeding behaviour traits per line. All meal characteristics were calculated per bird across the entire duration of the test. The four lines had been selected for different focus on growth traits.

	Line A	Line B	Line C	Line D
MLSN	12.2 ± 0.05	9.83 ± 0.04	15.3 ± 0.05	14.7 ± 0.05
MDUR (s)	444 ± 1.35	433 ± 1.36	331 ± 1.36	372 ± 2.06
MLSZ (g)	12.2 ± 0.05	13.3 ± 0.05	7.83 ± 0.03	7.27 ± 0.03
MNFD (s)	124 ± 0.64	84.6 ± 0.40	117 ± 0.65	141 ± 0.82
FDRT (g/min)	2.55 ± 0.01	2.50 ± 0.01	2.35 ± 0.01	2.28 ± 0.01
ADIT (g)	140 ± 0.26	124 ± 0.23	115 ± 0.17	101 ± 0.19
VISM	2.43 ± 0.02	3.00 ± 0.02	2.49 ± 0.01	2.16 ± 0.01
PRDF (min)	59.3 ± 0.19	57.8 ± 0.20	53.0 ± 0.26	53.6 ± 0.35
PRMF	0.720 ± 0.001	0.797 ± 0.001	0.642 ± 0.001	0.612 ± 0.001

Key: MLSN = number of meals per day; MDUR = meal duration; MLSZ = intake per meal; MNFD = non-feeding time in meal; FDRT = feeding rate; ADIT = average daily intake; VISM = visits per meal; PRDF = time per day spent feeding; PRMF = proportion of meal spent feeding

5.4.2 Heritabilities of traits

Table 2 shows the heritabilities for feeding behaviour and performance traits for each of the four lines and an overall mean across the lines. All traits were found to be ranging from moderately to highly heritable, with h^2 values varying from 0.231 (for body weight) up to 0.566 (for number of meals). Estimates for each trait varied slightly across lines, with the largest variation in average daily intake (0.258 to 0.391) and the smallest in lifetime feed intake (0.324 to 0.347). Although estimates per line differed significantly, there was no systematic variation across all traits with respect to growth rate, as illustrated in Figure 1. The traits with the highest heritabilities in all lines were: number of meals, intake per meal, feeding rate and proportion of the meal spent feeding.

Table 2: Heritabilities (± standard error) for feeding behaviour and performance traits for each line and the overall mean heritabilities across the four lines. Heritabilities and standard errors were estimated by the model, with overall mean across lines being calculated as a mean of the values estimated individually for each line. The four lines had been selected for different focus on growth traits.

Trait	Line A	Line B	Line C	Line D	Mean
MLSN	0.544 ± 0.018	0.536 ± 0.007	0.566 ± 0.020	0.560 ± 0.009	0.551 ± 0.004
MDUR	0.334 ± 0.015	0.298 ± 0.011	0.331 ± 0.013	0.243 ± 0.007	0.297 ± 0.014
MLSZ	0.517 ± 0.017	0.497 ± 0.010	0.517 ± 0.014	0.512 ± 0.008	0.510 ± 0.003
MNFD	0.291 ± 0.018	0.259 ± 0.011	0.380 ± 0.008	0.375 ± 0.014	0.326 ± 0.020
FDRT	0.502 ± 0.021	0.444 ± 0.009	0.558 ± 0.017	0.445 ± 0.007	0.487 ± 0.018
ADIT	0.391 ± 0.012	0.270 ± 0.010	0.333 ± 0.004	0.258 ± 0.007	0.302 ± 0.018
VISM	0.340 ± 0.010	0.375 ± 0.007	0.350 ± 0.005	0.317 ± 0.013	0.345 ± 0.008
PRDF	0.371 ± 0.012	0.314 ± 0.005	0.371 ± 0.015	0.314 ± 0.007	0.342 ± 0.031
PRMF	0.408 ± 0.009	0.404 ± 0.010	0.410 ± 0.013	0.492 ± 0.012	0.431 ± 0.042
BWT	0.231 ± 0.009	0.268 ± 0.008	0.294 ± 0.008	0.276 ± 0.009	0.267 ± 0.012
LFI	0.337 ± 0.008	0.324 ± 0.006	0.347 ± 0.007	0.334 ± 0.010	0.336 ± 0.009

Key: MLSN = number of meals per day; MDUR = meal duration; MLSZ = intake per meal; MNFD = non-feeding time in meal; FDRT = feeding rate; ADIT = average daily intake; VISM = visits per meal; PRDF = time per day spent feeding; PRMF = proportion of meal spent feeding, BWT = body weight, LFI = “lifetime” feed intake

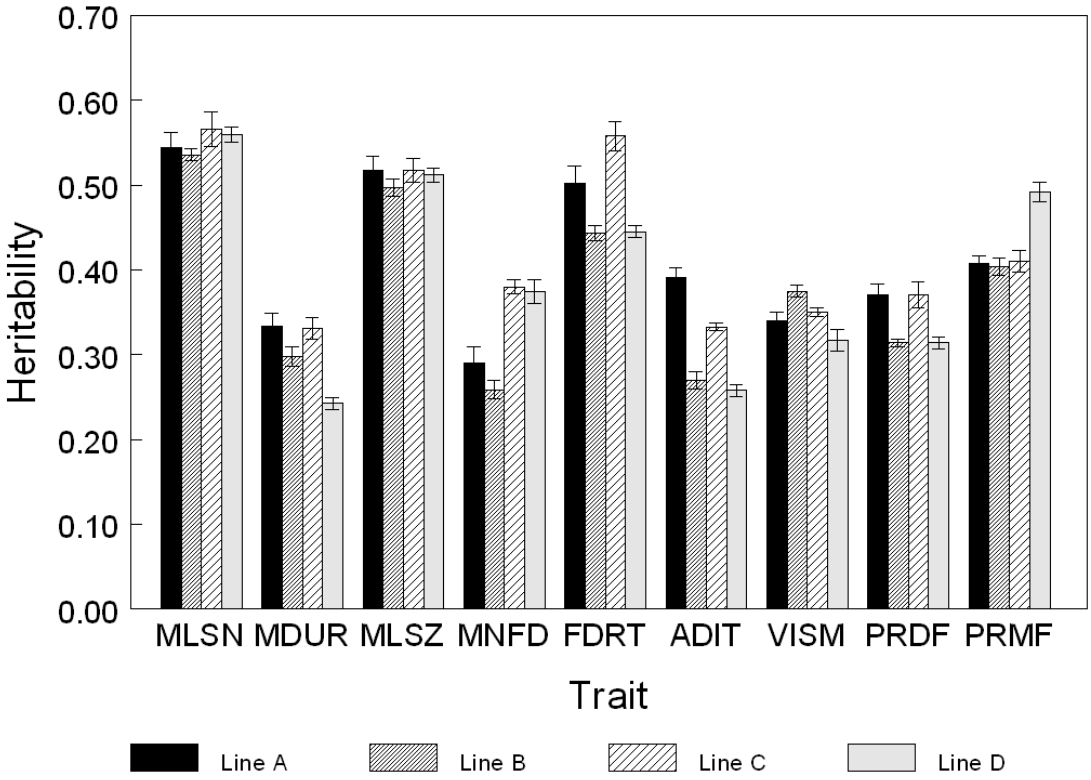

Figure 1: Comparison of heritabilities of feeding behaviour traits across lines, showing mean and standard error of each trait per line. For key to traits, see table 1.

5.4.3 Correlations between Feeding Behaviour Traits

Table 3 shows a matrix of the means and standard errors of phenotypic (above diagonal) and genetic (below diagonal) correlations between the feeding behaviour traits, combined across the four lines. Genotypic correlations tend to reflect phenotypic correlations, with traits with high phenotypic correlations also having high genotypic correlations (e.g. meal size and meal duration).

Very high genetic correlations (>= 0.85) exist between feeding rate, time spent feeding per day and meal duration, as well as between intake per meal and number of meals. Moderate to high correlations (± 0.5 to ± 0.85) were seen between non-feeding time per meal and a number of traits (see Table 3), as well as between meal size and proportion of the meal spent feeding. Other traits, such as average daily intake and meal duration, showed lower or no correlation, and were not significantly different from zero.

The most highly correlated traits indicate that birds that had larger meals tended to also have fewer meals per day. Also, birds with a higher feeding rate tended to spend less time per day feeding and also had shorter meals. The correlations of meal size or number of meals per day with feeding rate, meal duration or time spent feeding per day were much lower, but did show a trend towards larger meals being associated with faster feeding rate (correlation of 0.26).

Table 3. Matrix of phenotypic (above diagonal) and genetic (below diagonal) correlations between feeding behaviour traits across all four lines. Standard errors across the lines are given for each of the traits.

	MLSN	MDUR	MLSZ	MNFD	FDRT	ADIT	VISM	PRDF	PRMF
MLSN		-0.109 ± 0.045	-0.904 ± 0.005	0.419 ± 0.038	-0.173 ± 0.019	0.261 ± 0.044	0.010 ± 0.041	0.169 ± 0.025	-0.617 ± 0.029
MDUR	-0.093 ± 0.058		0.149 ± 0.043	0.496 ± 0.065	-0.669 ± 0.019	0.023 ± 0.014	0.362 ± 0.084	0.854 ± 0.014	0.081 ± 0.036
MLSZ	-0.961 ± 0.005	0.097 ± 0.052		-0.395 ± 0.035	0.179 ± 0.018	0.026 ± 0.032	-0.029 ± 0.039	-0.121 ± 0.025	0.607 ± 0.027
MNFD	0.463 ± 0.048	0.559 ± 0.052	-0.449 ± 0.053		-0.269 ± 0.037	0.036 ± 0.019	0.571 ± 0.017	0.328 ± 0.047	-0.751 ± 0.024
FDRT	-0.241 ± 0.036	-0.849 ± 0.017	0.256 ± 0.027	-0.484 ± 0.052		0.001 ± 0.038	-0.145 ± 0.058	-0.792 ± 0.019	-0.217 ± 0.041
ADIT	0.003 ± 0.050	-0.026 ± 0.066	0.202 ± 0.047	-0.086 ± 0.053	0.094 ± 0.079		-0.013 ± 0.025	0.157 ± 0.030	-0.020 ± 0.018
VISM	0.022 ± 0.033	0.471 ± 0.051	-0.005 ± 0.038	0.605 ± 0.029	-0.301 ± 0.039	0.001 ± 0.068		0.163 ± 0.058	-0.360 ± 0.036
PRDF	0.230 ± 0.037	0.903 ± 0.013	-0.212 ± 0.033	0.474 ± 0.048	-0.952 ± 0.005	0.092 ± 0.039	0.330 ± 0.049		0.183 ± 0.045
PRMF	-0.661 ± 0.013	0.181 ± 0.061	0.652 ± 0.018	-0.734 ± 0.022	-0.142 ± 0.041	0.101 ± 0.029	-0.237 ± 0.076	0.155 ± 0.053	

Key: MLSN = number of meals per day; MDUR = meal duration; MLSZ = intake per meal; MNFD = non-feeding time in meal; FDRT = feeding rate; ADIT = average daily intake; VISM = visits per meal; PRDF = time per day spent feeding; PRMF = proportion of meal spent feeding

5.4.4 Correlations with Performance Traits

The correlations of the feeding behaviour traits with the performance traits for all lines are illustrated in Figure 2. All the correlations were low (ranging from ±0.02 to ±0.18) with the exception of average daily intake, which showed moderate to high correlations with lifetime feed intake (up to 0.716, for line A). For most traits, there were broadly similar correlations between the lines although more variation was seen with body weight, with line C showing opposite direction of correlation with feeding rate, number of visits per meal, time spent feeding per day and proportion of meal spent feeding. However, the correlations were still very low in all lines, and some estimates had quite large standard errors.

(a) **(b)**

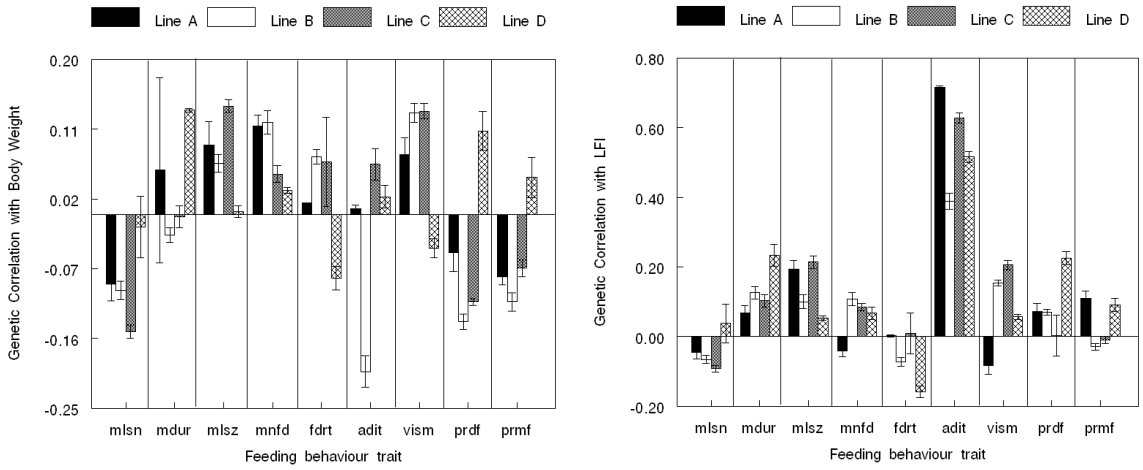

Figure 2: Genetic correlations (and standard errors of estimates) of feeding behaviour traits with (a) body weight and (b) lifetime feed intake for each of the 4 lines.

5.4.5 Feeding Strategies

Figure 3 shows the distribution of meal sizes for birds with the lowest (i.e. best) 25% FCR across all line A birds, plotted against body weight and feed intake. Both graphs show a slight trend towards increase in meal size with increased body weight and increased feed intake, but a large variation exists between individual birds (r^2 is 0.07 and 0.04 for body weight and feed intake respectively). The very high phenotypic correlation between meal size and number of meals per day of -0.904 (table 3) indicates that birds which take large meals tend to also take fewer meals per day. Figure 3 shows that birds which have the best FCR achieve this using a variety of feeding strategies, ranging from mean meal sizes of approximately 5g (with birds having around 25 meals per day) to 25g (with birds having approximately 5 meals per day). This range of meal sizes was the same if all line A birds were considered.

(a) **(b)**

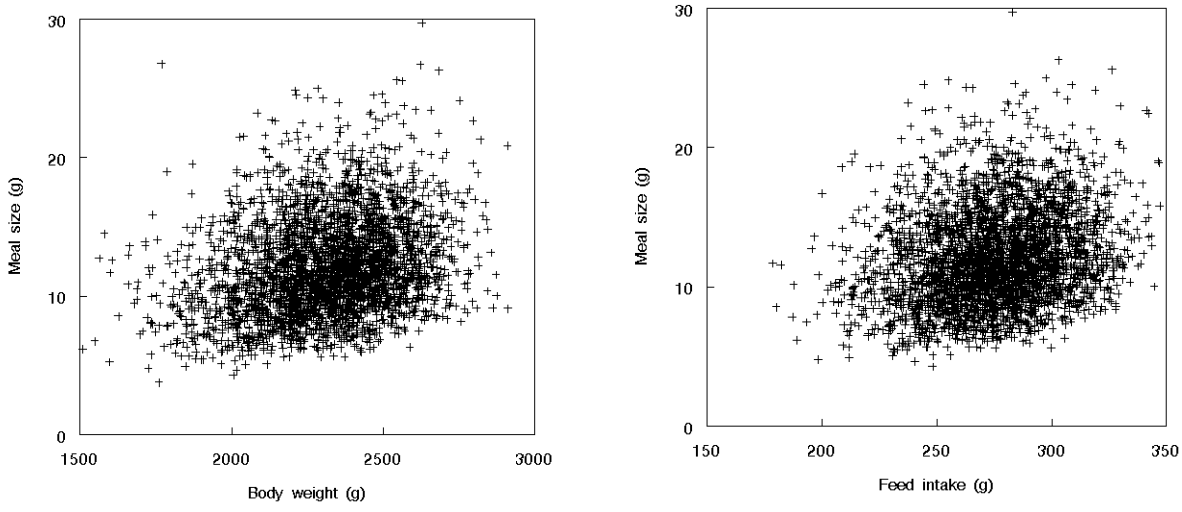

Figure 3: Scatterplot of adjusted meal size against (a) body weight and (b) feed intake for birds in the top quartile for FCR from line A.

The distributions of meal size EBVs against body weight EBVs and lifetime feed intake for one hatch of birds in line A are shown in Figure 4. If a reference point of the mean body weight for all the birds is used (represented as an EBV of 0), there is a range of meal size EBVs from highly negative to highly positive EBVs (figure 4a). Thus although there is a clear trend that birds with greater EBVs for BWT will have a greater EBV for meal size (the genetic correlation is 0.104), it is possible to select birds which take very small or large meals which achieve the same body weight gain. Similarly, large variation is seen in meal size EBVs when compared to lifetime feed intake (figure 4b). If the mean value reference point is used again, there are birds with approximately -8g up to +8g variation on the mean meal size which achieve the same feed intake. Both figures 4a and 4b show that there is potential for selection of birds with different feeding strategies whilst maintaining current selection targets for body weight gain and lifetime feed intake.

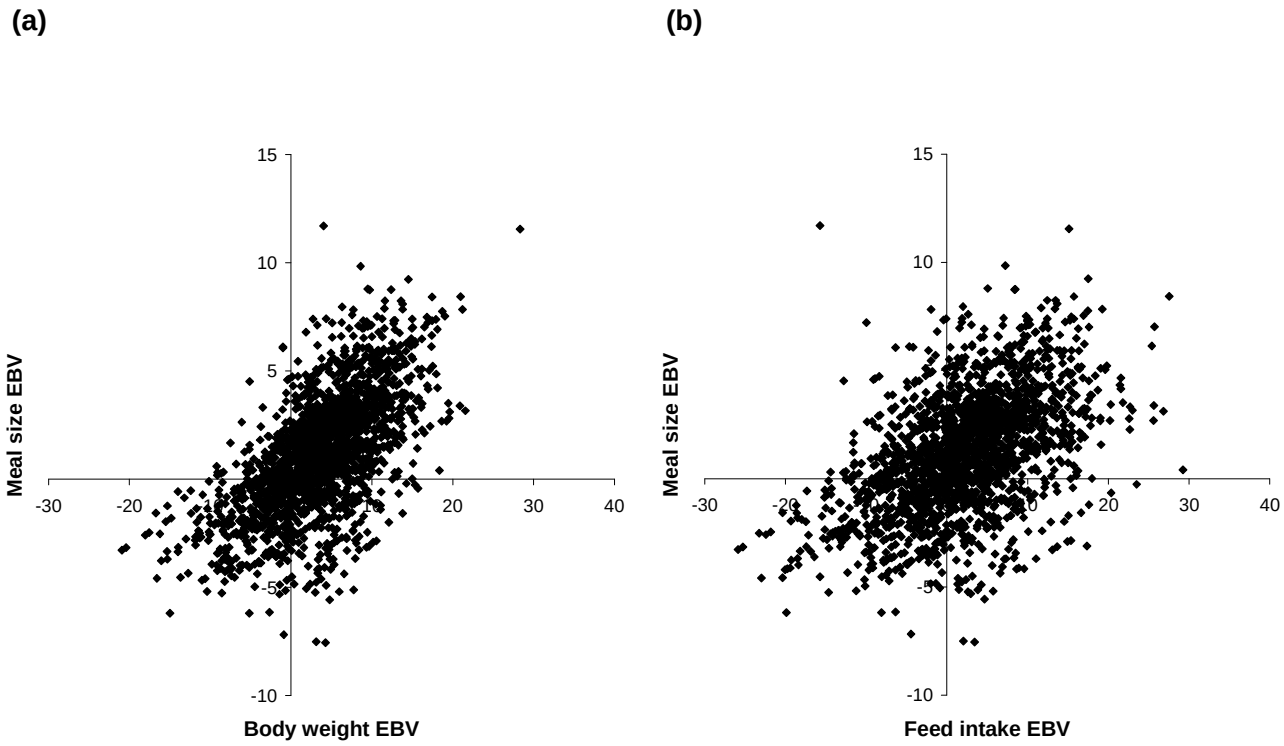

Figure 4: Scatterplot of estimated breeding values (EBVs) for meal size against (a) body weight (at 35 days) and (b) lifetime feed intake for a hatch of birds from line A.

5.5 Discussion

Selection for improved feed conversion has had a large impact on the poultry industry, allowing reduction of feed costs whilst maintaining body weight gain and high levels of bird welfare (McKay 2007). Identification of new traits relating to feeding behaviour may aid in continued progress in the poultry industry and allow optimisation of selection strategies for feed efficiency. In this study, we have used meal-based feeding behaviour traits to identify differences in feeding strategies between individuals to estimate both the impact of selection for improved FCR on feeding behaviour as well as the potential for future selection for specific feeding behaviours in breeding programmes.

At a phenotypic level, it has been previously shown that the organisation of feeding behaviour into bouts in these four lines of broilers is similar, despite differences in selection intensity and performance goals (Howie et al., 2009b). Birds from all four lines were found to feed in distinct bouts, and showed an increase in the probability of starting to feed with increasing time since the last feed, which is consistent with the concept hunger and satiety controlling feeding behaviour (Tolkamp and Kyriazakis 1999). At a meal-based level, patterns of feeding behaviour do differ between the lines, with the two faster growing lines A and B having fewer meals than the two slower growing lines (Table 1). These meals were on average, larger and longer in duration than for lines C and D. The faster growing birds also tended to spend slightly more time feeding per day and had a higher daily intake.

However, these phenotypic differences between lines were not reflected in large variations in either heritabilities (Table 2) or genetic correlations (Figure 2) across the lines, indicating that the relationships between individual feeding traits and between feeding behaviour traits and the

component traits of FCR are highly conserved across the lines, despite differences in selection focus for growth. Genetic correlations between the nine feeding behaviour traits ranged from 0.001 (between average daily intake and number of visits) to -0.961 (between number of meals and intake per meal), and were similar to those found in the literature for visit-based feeding behaviour in other species (e.g. pigs - Kalm et al., 1996; Labroue et al., 1997). High correlations between traits suggest that they are under the control of the same genes and thus selection for one of these traits would have a large effect on the highly correlated trait. Conversely, a very low genetic correlation indicates that there would be little impact of selection on the other trait. All feeding behaviour traits, except for average daily intake, showed very low correlations with all the components of FCR, i.e. the traits were not strongly linked (Figure 2). These low genetic correlations between feeding behaviour traits and the components of FCR indicate both that previous selection for FCR has had little impact on feeding behaviour in broilers and that selection for specific feeding behaviours is expected to have a low impact on FCR.

Not all traits in this study showed similar heritabilities or correlations to those previously found in the literature for other species subjected to similar selection intensities; there are no comparable studies in birds on these traits. For example, both Schulze et al (2003) and Hall et al (1999) found high correlations between intake per feeding event and event duration in pigs, whereas we find a much lower correlation for chickens (see Table 3). This may be due to most previous studies having used visits as the unit of analysis, and may also reflect a true species difference, as these were carried out in pigs or cattle (e.g. Von Felde et al., 1996; Hall et al., 1999; Schulze et al., 2003; Robinson and Oddy 2004). We have shown that visits to feeders are not meals in themselves but that such visits are clearly clustered and can be grouped into meals

on the basis of suitable meal criteria (Yeates et al., 2001; Howie et al., 2009a, 2009b). It is, therefore, inappropriate to use the term "meal" for visits, or clusters of visits grouped together on the basis of an arbitrary meal criterion. It is evident from these analyses that the magnitude of heritabilities of 'meal' traits and of genetic correlations between two 'meal' traits or between a 'meal' trait and a performance trait can be affected considerably by the meal definition. This would have a large impact on the estimated values of feeding behaviour traits and may explain the large range in heritability and genetic correlation estimates across studies. This emphasizes the importance of using non-arbitrary meal criteria that are based on direct analysis of animal behaviour, for genetic as well as other types of analyses (e.g. Zorrilla et al., 2005)

In addition to assessing the impact of past selection on feeding behaviour, this study aimed to identify the potential for selection for different feeding behaviours without compromising past or future improvements in performance traits. The need to breed birds with different feeding strategies may arise in different environmental conditions, thus optimising selection strategies to manage the genotype by environment interaction. For example, in less than optimal conditions where feed is not readily available, birds that take large but infrequent meals may be more suited than those that feed frequently. Conversely, birds fed on a poor quality but readily available diet might benefit from feeding more often and thus a bird which takes frequent short meals may be more suited for such an environment.

Figure 3 shows that birds with the best FCR use the full range of average meal sizes to achieve their feed intake when compared to the whole population of birds in the study. There is a slight trend towards increased body weight with increased meal size, but the correlation is low

(0.104, Figure 2). The range of EBVs for the whole population of birds shows that there is enough variation to be able to select birds that have large or small meal sizes, whilst maintaining or further improving body weight and lifetime feed intake. Due to the high correlation between meal size and number of meals, it would only be necessary to use one of these traits in a selection matrix. Thus there is potential for selection of birds based on feeding behaviour traits, which may be suited to specific environmental conditions, thereby improving welfare.

5.6 Conclusions

This study shows that feeding behaviour traits in all four broiler lines have mostly similar heritabilties and genetic correlations. Low genetic correlations with performance traits indicate that current selection programs have had little impact on feeding behaviour, and that future selection for favourable feeding behaviours would be possible without affecting FCR goals. Analysis of the feeding behaviour of birds with the best feed conversion ratios showed that a range of feeding strategies are used to achieve the same FCR, thus offering the potential for selection for birds with specific feeding strategies.

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CHAPTER 6

Genome-wide analysis identifies regions associated with feeding behaviour in broilers

6.1 Abstract

Selection for performance traits such as body weight gain can have correlated, and sometimes detrimental, effects on other traits. Previous analysis revealed low correlations between feeding behaviour traits and body weight, indicating little effect of past selection on feeding behaviour. Genetic variation between individuals, estimates from phenotypic records and pedigree information, gives the potential for selection for different feeding behaviours, which may be suited to different environmental conditions, without affecting performance traits. However, the control of feeding behaviours at a genomic level has not previously been analysed and will provide much information for understanding relationships with other traits, as well as being a useful tool for improving selection accuracy. In this study, we used phenotypic records from 61,835 female broiler birds to identify which SNPs were most significantly associated with feeding behaviour. These birds were the offspring of 200 genotyped sires. Association analysis was performed on the adjusted offspring means of these sires for nine feeding behaviour traits and two performance traits. A SNP panel of 12,046 SNPs was used and association was performed on each individual SNP for all the traits. Significant associations were found across the genome, with highly correlated traits, such as meal size and number of meals per day, showing links with the same regions. Feed intake was found to have significant associations with SNPs across 4 chromosomes, independent of body weight, indicating that these regions may have an important role in the control of feed intake. With the identification of the areas of the genome associated with feeding behaviour, it will be possible to predict the effects of selection for other traits on the expression of

feeding behaviour at a genotype level, and the impact of any future selection for feeding behaviours on other production goals.

6.2 Introduction

Improvement in feed conversion ratio has led to vast reductions in the amount of feed needed to rear broiler chickens. Since 1975, feed requirements have dropped from around 20 million tonnes per million tonne of meat to just 8.5 million tonnes (McKay 2007). As feed is such a high cost in production, methods to further improve feed efficiency would have a massive economic impact. Within lines of broilers, individuals have been found to vary in their short-term feeding behaviour, with some taking less frequent, larger meals than others to achieve the same feed intake (Howie et al., 2009b). These feeding behaviours were found to only have low genetic correlations with body weight and so there is potential for selection for specific feeding behaviours, which may be suited to perform better in particular environmental conditions, whilst continuing improvements in body weight gain.

Currently, selection programmes are based on estimates of breeding values which are calculated using the phenotype of the animal and information derived from the pedigree (Falconer & MacKay 1996). Understanding of the genetic control of traits would greatly increase the accuracy of these estimated breeding values, as well as allowing direct selection of individuals carrying particular alleles associated with a desirable trait (Goddard and Hayes 2009). As feed intake is such an economically important trait, genomic selection for feeding behaviour may also provide greater accuracy of predictions of individual genetic merit and thus increase profitability.

Chapter 6 – Associations for STFB with SNPs

Feeding behaviour of individual birds is difficult to measure without the use of specialised equipment, such as electronic feed stations. The identification of genomic markers would allow marker-assisted selection and estimation of genetic merit for such traits, without the need for measurements of feeding behaviour to be recorded for all individuals (Dekkers et al., 2004).

With the mapping of the chicken genome (International Chicken Polymorphism Map Consortium 2004) and the high abundance of SNPs across the genome, it is now possible to investigate which regions of the genome are associated with particular traits. No previous studies have looked at genomic links with short-term feeding behaviour traits in broilers, and therefore the primary aim of this study was to determine which regions of the genome showed the most significant associations with feeding behaviour in broiler chickens, to increase understanding about the control of feeding. Phenotypic records of feeding behaviour were available from individual female birds from a pedigreed line of broiler chickens, covering a period of approximately three years. Genotypic data of the sires and the SNP panel were provided by Aviagen Ltd as part of their Genomics Initiative Project. As a secondary aim, SNPs linked to body weight and feed intake were also identified to assess the degree of pleiotropy between these and the feeding behaviour traits, and thus the potential impact of selection for one trait on the others. Regions of the genome significantly associated with feed intake and body weight were also identified.

6.3 Materials and Methods

6.3.1 Data source

Phenotypic records of feeding behaviour traits were available for 61,835 female broiler chickens. The line of birds used in the present study was the same as line B in Howie et al (2009b), which obtained an average body weight of 2.1 kg by the age of 35 days. Only one line was used in this study due to high similarities in correlations with body weight and feed intake traits across the four lines studied previously. Line B was chosen as the most genotypic data were available for this line. The phenotypic data were collected during a period of three weeks for individual birds, group-housed in pens with 8 electronic feeders per pen, as described in detail in Howie et al (2009a). In the present study, the feeding behaviour records covered a period of approximately 3 years. Meal characteristics were estimated using meal criteria derived by a truncated normal method, outlined in Howie et al (2009a). These were then pre-adjusted for the effect of body weight prior to genetic analysis, using a correction factor estimated from regression of body weight on the phenotype of the trait. Genetic variances and heritabilities were obtained from an individual animal model BLUP using PEST and VCE software, with a pedigree of 341,847 animals, with body weight and feed intake records covering a period of 5.5 years. Overall, 3 production and 9 feeding behaviour traits were included in the analysis. These were body weight (BWT) at 35 days, “lifetime” feed intake (LFI) and adjusted feed intake (lifetime feed intake adjusted for body weight) and the means of: number of meals per day, intake per meal, meal duration, number of visits per meal, daily feeding time, proportion of the meal spent feeding, non feeding duration within a

meal and daily intake. “Lifetime” feed intake refers to total feed consumption during the three-week experimental period when the birds were housed with the electronic feeders and was the only trait not adjusted for body weight.

6.3.2 Data analysis

The pedigree information was available for 200 sires and their offspring, with a mean offspring number of 173 birds per sire (minimum of 17 and maximum of 391). Adjusted offspring means were calculated for each of the traits per sire to take into account fixed effects of mating group, hatch week, sex and pen**,** and half the breeding value of the dam. Offspring means were used as phenotypic values of feeding behaviour traits were not available for the sires. Regression analysis was used to calculate the adjusted heritiabilities for each trait, using the values for phenotypic and genetic variation estimated from the BLUP analysis. Adjusted heritabilites were calculated as: $0.25\ V_a$ / $(0.75\ V_a + V_c + V_e)$.

6.3.3. SNP panel

Each of the sires had been previously genotyped and the alleles present at each SNP locus were categorised as 0, 1 or 2, depending on the number of copies of the most common allele present (as defined for the Illumina SNP chips used). Association analysis was performed on an individual SNP basis for a 12,046 panel of SNPs which were distributed across the entire genome. 3000 of these SNPs were the same as those from an initial panel developed from the USDA project (USDA-ARS, Avian Disease

and Oncology Laboratory, East Lansing, MI). The other SNPs were chosen to increase density and improve the distribution of SNPs across the entire genome. A total of 8,947 SNPs, with allelic frequency greater than 0.05, were used for the association analysis. This gave an average distance of 1 SNP per 0.40 cM across the genome.

The model used for the association analysis was:

$$Y = \mu + Xb + Zu + e$$

Where: Y = the n x 1 vector of adjusted progeny means for the trait for n sires, μ = population mean, b = fixed SNP allelic substitution effect, X = n x 1 vector of no. of copies of allele 1, u = n x 1 vector of random sire polygenetic effects ($u \sim N(0,\sigma^2 u_i)$), Z = incidence matrix, e = n x 1 vector of random residuals corrected for p ($e_i \sim N(0,\sigma^2 e_i/p)$) where p = number of offspring on which adjusted mean of sire was calculated.

This model was fitted using linear regression, with b being the regression co-efficient. The method for SNP association was linkage disequilbrium (LD) mapping (Zhao et al., 2007) and was fitted using in-house software provided by Aviagen. Significance levels were calculated for each individual SNP for every trait using F tests, and the resulting p values were used to identify the areas of the genome most significantly associated with each of the traits. The p values were checked for uniformity using graphical methods, to establish whether the differences in heritabilities between the traits were causing

distortion of the p values in certain areas of the genome which could greatly affect the significance of associations with SNPs (Hassen et al., 2009).

6.4 Results

6.4.1 Association analysis

Association analysis showed significant associations ($p<0.05$) of feeding behaviour traits with SNPs on all chromosomes. Table 1 shows the number of significant associations for each of the feeding behaviour traits.

Table 1: Number of SNPs across the genome significantly associated with each of the feeding behaviour traits at the p <0.05, p <0.01 and p < 0.001 level.

	Significance level		
Trait	**p < 0.05**	**p < 0.01**	**p < 0.001**
Daily intake	409	64	3
Feeding rate	445	73	6
Meal duration	543	108	24
Number of meals	481	96	13
Meal size	477	94	12
Non-feeding duration	407	82	12
Daily feeding time	530	113	13
Proportion of meal feeding	425	81	5
Visits per meal	503	112	6

Table 2 summarises the chromosomes which were found to have SNPs with the most significant level of association ($p<0.001$). As can be seen from figures A1 to A3 in the thesis appendix, these regions with the most significant levels of association were often surrounded by SNPs with lower significance levels ($p<0.05$ and $p<0.01$). These 3 figures show the association results for SNPs across the whole genome, with the highest significance levels being shown as red ($p<0.001$), purple ($p<0.01$) and yellow ($p<0.05$) respectively for each of the traits.

Table 2: Chromosomes which show the most highly significant associations ($p<0.001$) with feeding behaviour traits

Trait	Chromosomes with significant SNP associations
Daily intake	7, 11
Feeding rate	3, 4, 9, 11, 13
Meal duration	1, 3, 4, 5, 7, 11, 14, 20, 27, Z
Number of meals	1, 2, 4, 19, 27, Z
Meal size	1, 2, 7, 19
Non-feeding duration	1, 2, 5
Daily feeding time	2, 4, 5, 11, 28, Z
Proportion of meal feeding	2, 3, 5, 6, 25
Visits per meal	1, 2, 3, 5, 28

6.4.2 Checking the results for bias in p values

Figure 1 shows the distribution of p values for all the SNPs for an example feeding behaviour trait (number of meals per day). The p value estimates show a uniform distribution across the range of p values, indicating that there is not a bias towards high or low significance levels across the estimates. All the other traits showed a similar uniform distribution (not shown). Therefore it seems that the heritability adjustments, carried out before the analysis, were successful in preventing any bias.

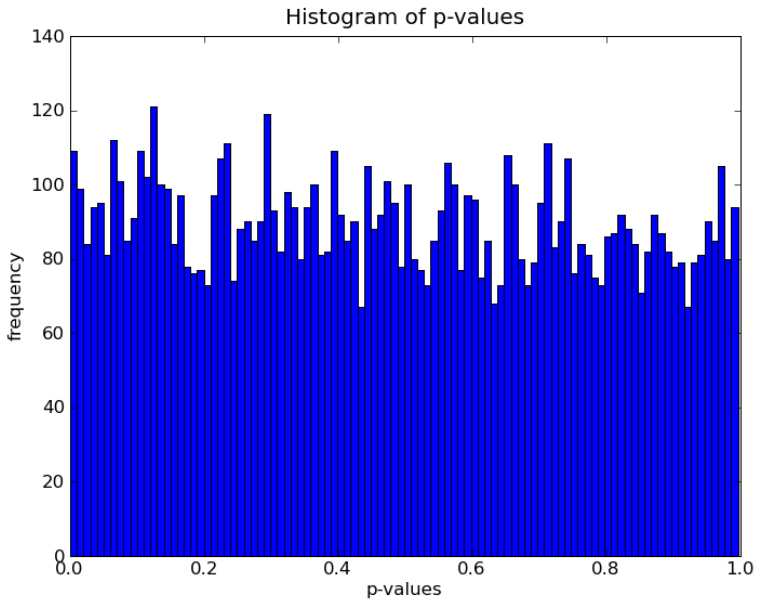

Figure 1: Distribution of p value estimates for association analysis between SNPs and number of meals. Bars show the number of incidences of each p value (bin width = 0.01) for all SNPs.

6.4.3 Genetic correlations between traits

Genetic correlations between the feeding behaviour and performance traits were previously estimated (Chapter 5). High correlations were observed between meal size and number of meals per day (-0.961 ± 0.005), as well as between meal duration and feeding rate (-0.849 ± 0.017) and between meal duration and daily time spent feeding (0.903 ± 0.013). A high correlation was also seen between two of the performance traits, body weight and lifetime feed intake (0.787 ± 0.008). All other traits showed much lower correlations. Similarly, the correlations between all of the feeding behaviour traits (except average daily intake) and the performance traits were very low, and some were not significantly different from zero (e.g. feeding rate, daily feeding time and visits per meal with body weight).

In general, the highly correlated feeding behaviour traits were found to have significant associations with mostly the same SNPs, indicating a possible pleiotropic effect of genes in these regions of the genome (e.g. meal size and number of meals shown in Figure 2 below). In the thesis appendix, Figure A1 shows the significance level of SNPs across the genome for the highly correlated traits of meal size and number of meals. The coloured dots on the figure show the significance of associations of the traits with SNPs, and nearly all SNPs that show significant associations with one of the traits do so with the other. This was also found to be the case for feeding rate, meal duration and proportion of the meal spent feeding (see appendix - Figure A2).

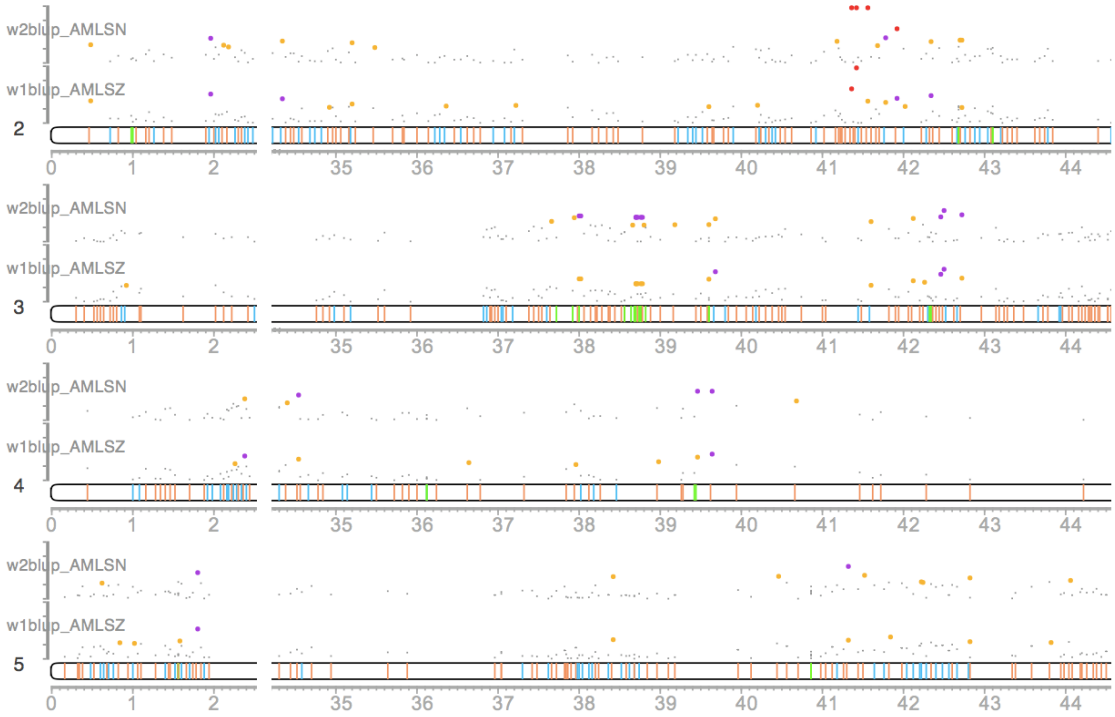

Figure 2: SNP associations for number of meals (MLSN) and meal size (MLSZ). Dots on the figure show the level of significance of association at each SNP location (grey: p>0.05, yellow: p<0.05, purple: p<0.01, red: p<0.001). Bars indicate location of the SNPs (orange: SNP outside known gene, light blue: SNP within known gene, green: SNP within gene previously found to be associated with feed intake). Associations are shown for parts of chromosomes 2, 3, 4 and 5 to illustrate that both traits show significant associations with the same SNPs (for example, between 41 and 43Mb on chromosome 2).

The final figure in the appendix, Figure A3, shows the SNP associations between the 3 performance traits, lifetime feed intake (with and without adjustments for body weight) and body weight. Most chromosomes have SNPs with significant associations with both

body weight and lifetime feed intake. However, few areas showed significant associations with both adjusted and lifetime feed intake but not body weight, which may be possible candidate regions for investigation of genes controlling feed intake (e.g. Figure 3 below).

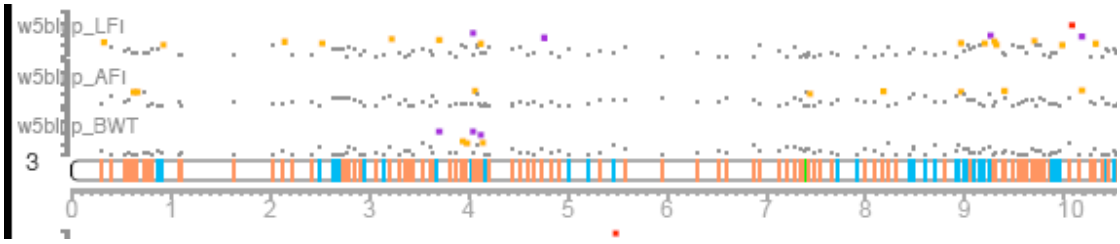

Figure 3: An example of SNPs significantly associated with the feed intake traits and not body weight. The region around 9 to 10Mb shows a number of significant associations (red, yellow and purple dots) with AFI and LFI (adjusted and lifetime feed intake).

The locations of the SNPs associated with performance traits were broadly different to those found for the feeding behaviour traits, which could be expected as we had previously found a low genetic correlations (Chapter 5, Figure 2). For example, most of the significant SNPs shown on Figure A3 for feed intake are not significantly associated with any of the feeding behaviour traits in Figures A1 or A2.

6.5 Discussion

Genome-wide association analysis has allowed us to identify regions of the genome with a link to feeding behaviour traits in broiler chickens. As feed intake is such an economically important trait, and feeding behaviour is the temporal structure of feed intake (Blundell et al., 1985), understanding of the genomic control of feeding behaviour may be beneficial in improving feed efficiency. In addition it has been shown that feeding behaviour traits are not correlated with many of the performance traits of interest, opening up the possibility that selection may be based on feeding behaviour strategies without affecting any of the performance targets. Feeding behaviour traits in broilers tend to be highly heritable, thus making them good candidates for selection (Chapter 5). All traits, except average daily intake, showed low genetic correlations with both body weight and lifetime feed intake, indicating that they would not be good predictors for either trait. However, the low correlations mean that feeding behaviour traits could be successfully selected for, without having detrimental effects on body weight gain.

Analysis of significance levels of associations with SNPs indicates that there are a large number of areas across the genome that are highly associated with feeding behaviour. Figures A1 and A2 in the appendix show that these regions are generally the same for highly correlated traits, indicating both traits are controlled by the same genes. These figures allow identification of target regions for further investigation into specific genes which are highly associated with short-term feeding behaviour.

Similarly, comparisons of lifetime feed intake, adjusted feed intake and body weight suggest areas on chromosomes 1, 2, 3 and 7 may be important in the control of feed intake in this line of broilers. If Figure A3 is compared with figures A1 and A2, it is evident that generally the regions of the genome significantly associated with feeding behaviour traits are broadly different to those associated with the performance traits, which agrees with the low genetic correlation seen between the feeding behaviour and performance traits.

As previously stated, no previous studies have looked at SNP associations with feeding behaviour in broilers. However, some studies have identified quantitative trait loci (QTL) associated with feeding behaviour traits in pigs. Zhang and colleagues (2009) found significant QTLs for number of visits to a feeder and feeding rate on chromosomes 7 and 9. Unfortunately it is not yet possible to compare these results with chicken chromosomes. A few studies have investigated QTLs for feed intake and feed efficiency in poultry. In chickens, Abasht et al (2006) provided a review of all identified QTL, with QTLs for feed intake having been found on chromosomes 1, 2, 4 and 9 in two different studies (van Kaam et al., 1999, Tuiskula-Haavisto et al., 2004). In other species, chromosome 2 seems to have significant associations with feed intake (e.g. cows -Sherman et al., 2008 and pigs - Houston et al., 2005). As yet, the control of feed intake and feeding behaviour at the genomic level is not fully understood, but information from SNP and QTL associations can be used for predicting breeding values

and increasing accuracy of selection. As more information becomes available, rapid improvements in accuracy can be achieved.

The next stage of analysis from this study would be to use this information to investigate which specific genes, and alleles of these genes, are linked to STFB in order to use the SNP for selection purposes (Falconer & MacKay 1996). If specific alleles can be definitely associated with a particular phenotype of the trait, breeding merit could then be estimated by genotyping for the presence or absence of the allele, thus not requiring specialised feeding equipment to record behaviour. As feeding behaviour is highly heritable (Chapter 5), rapid progress can be made in selection for feeding behaviours linked to favourable production goals or for specific short-term feeding behaviours themselves.

6.6 Conclusions

I have identified regions of the genome significantly associated with short-term feeding behaviour and performance traits in a line of broiler chickens. Highly correlated traits showed significant associations with SNPs in the same areas of the genome, as would be expected. Analysis of feed intake, pre-adjusted for body weight, has suggested areas of the genome which are likely to be involved in the control of feed intake in broiler chickens, and which are thus candidates for further investigation.

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CHAPTER 7

General Discussion

One of the primary focuses for a breeding programme is the continued improvement in feed efficiency, whilst maintaining or improving other traits such as growth and breast meat yield. Improvement in feed efficiency relies on individual variation in feed intake to enable selection for the most efficient birds. Breeding companies often measure individual feed intake in order to calculate feed conversion ratios, but currently do not use any data on individual feeding behaviour itself. Feeding behaviour data provides insight into how birds which achieve the same daily intake differ in terms of organisation of that feed intake into meals. It may be that more efficient birds tend to follow a similar organisation of meal patterns, be it taking many short meals, which could allow maximum digestion of feed, or taking a few, large meals, which would lead to less energy expenditure and thus may be a more efficient method of feeding. This study has used these data to investigate the structure of short-term feeding behaviour in broiler chickens, both within and between lines and its potential for use of individual variation in this feeding behaviour in breeding programmes.

Firstly, the structure of short-term feeding behaviour in broiler chickens was investigated. Although feeding behaviour in broilers has been analysed in the past (e.g. Masic et al., 1974, Barbato et al., 1980) none of the studies have used a biologically relevant method for grouping the unit of measurement of feeding behaviour into meals. Most studies have used criteria of around 2 minutes, estimated using the log-survivorship method (e.g. Masic et al., 1974, Duncan et al., 1970), which is much shorter than those estimated in the current study. Therefore, the daily number of meals

found in the current study was considerably less than those found by studies where a 2 minute meal criterion was used. For example, Masic and colleagues (1974) found a mean of 49.5 meals per day, whereas using the new models in this study, I found an average of 13 across all four broiler lines (Chapter 3, Table 2). As discussed by Tolkamp and Kyriazakis (1999), differences in criteria values for grouping visits have a large effect on the resulting size and number of the groups, thus making meaningful comparisons between studies very difficult. Current models based on the idea that hunger and satiety control short-term feeding behaviour, such as those by Tolkamp, Yeates and colleagues (discussed in Chapter 1) rely on there being readily identifiable and separate distributions of within- and between-meal intervals. However, as illustrated by figure 1b in chapter 2, broilers were found to have a different distribution of within-meal intervals to those found previously in other livestock species (e.g. Tolkamp et al., 1998). This distribution was not easily modelled, and the log-normal distributions used in those previous models were unsuitable for describing the within-meal distribution of interval lengths in broilers. Thus in order to analyse the data, I adapted the models for use on the broiler data (Chapter 2).

The log-transformed between-meal distribution of interval lengths in broilers was found to follow a log-normal distribution (Chapter 2, Figure 1), and a truncated model of this distribution was fitted to the data as a representation of only the interval lengths between meals. This truncated log-normal model, and a second model based on the changes in probability of starting to feed with time since last feeding were found to give very

similar estimates in meal criteria (Chapter 2, Table 2). When tested with data from previous experiments with cows, the two new models were also found to give similar estimates to when Yeates' model was applied (Chapter 2, Table 3). Therefore, not only are these new models applicable for analysis of broiler data, they can also be used reliably on data that do have a recognisable distribution of within-meal intervals. Further analysis of the within-meal distribution of interval lengths in broilers showed that if separated into visits to the same and to different feeders (Chapter 4, Figure 3), then if only the intervals between visits to different feeders were considered, the log-transformed distribution of intervals was similar to those seen in cows (Tolkamp et al., 1998, Yeates et al., 2001).

At a practical level, the new models developed in chapter 2 are applicable at a flock and a strain level, depending on the amount of data available. A model involving breaking the data down into intervals to different and the same feeders, as was done in chapter 4, would be less easily utilised in a breeding programme, as it requires more manipulation of the data. Analysis in chapter 4 showed that the truncated normal model is applicable across poultry species and it could easily be automated for use in a breeding programme. Chapter 3 illustrated that current meal criteria estimates ranged from 17.5 min to 20min in the four lines analysed, and therefore it is possible that they may change over time with selection pressures. As STFB may be genetically linked to another selection goal not investigated in this thesis (e.g. breast meat yield), it is possible that improvement in this linked trait may cause alterations in STFB and meal structure. Any inaccuracies in

the meal criterion used will lead to visits not being correctly grouped into meals and thus reduce the relevance of meal-based STFB analysis on this data. Therefore, these values should be reviewed periodically to reflect any changes in short-term feeding behaviour which occur during this time period.

The birds of all four lines were housed in the same environmental conditions, and the experimental conditions for measuring short-term feeding behaviour were the same, which allowed accurate comparisons between lines, without compounding environmental effects. Unfortunately, in the across species comparison in chapter 4, the same environmental conditions couldn't be applied to all 3 species. As data for this study were acquired from commercial breeding programmes with different experimental set-ups and ages for feed conversion testing, it was not possible to standardise experimental conditions across the three species. However, despite these differences, all the species showed similar bouted feeding behaviour, and short-term feeding traits could be estimated. Ducks were, however, found to spend much less time per day feeding than either the broilers or the turkeys, and had a considerably faster feeding rate than broilers, despite similarities in the size of the birds (Chapter 4, Table 3). This may be due to the way in which ducks feed, having large bills which allows them to take in more feed at a time than a similar-sized chicken. From a production consideration, it would therefore be possible to have a higher density of birds per feeder for ducks, as compared to the two other species, without compromising free access to feed, as the birds spent less time feeding per day and therefore occupancy of the feeders were much lower.

At a genetic level, short-term feeding behaviour traits were analysed along with the component traits of FCR, to estimate both the heritability of traits and their relationship with feed conversion production goals (Chapter 5). In the models, the components of FCR, i.e. body weight and feed intake, were used instead of FCR itself as it provides more information than the relationship with FCR. Currently, some broiler breeding programmes are moving away from selection based on breeding values of FCR specifically, but are using the two component traits instead. Direct selection for improved FCR will have an impact on both body weight and feed intake, whereas if the traits are considered independently then increases in body weight can be achieved with a lesser impact on feed intake and appetite. Thus selecting on individual components allows increased control of the effects of selection and can lead to increased progress in FCR (Koerhuis and Hill 1996).

On a similar theme, meal size and number of meals could be considered to be the components of feed intake, as daily intake is achieved by these two factors (Meguid et al., 1998). Therefore it could be possible to select for birds with the same feed intake which differ in their feeding behaviour (for example, some birds taking lots of small meals, whereas others take a few large meals). The traits were found to be a lot more heritable than either feed intake or body weight. If there had been a high genetic correlation with either of these traits, the feeding behaviour trait could then be used for selection instead of the less heritable feed intake or body weight. However, this was not the case, but may well be the case with some of the other performance traits. Therefore a

future direction of analysis from this study should be to analyse the genetic correlations of short-term feeding behaviour traits with other performance traits which show low heritability, or are difficult to measure (such as carcass traits – Dekkers 2004). As most breeding companies select for a variety of targets, this will also allow assessment of the impacts of selection for these other goals, such as increased egg production and fertility, on feeding behaviour.

As discussed in Chapter 5, it may be desirable to select for specific feeding behaviours to suit different environmental conditions. Future work should be to estimate the response to selection for different feeding strategies, to assess how quickly differences in feeding strategy between flocks can be achieved. Chapter 2 illustrated that the genetic lines show differences in short-term feeding behaviour, and that there is still variation between individuals within the line. It would also be interesting to compare the current findings in broilers with those from a control line, which had not experienced the intensive selection for increased growth rate and body weight. Aviagen keeps a control line of birds which have been maintained at the level of selection that was achieved in 1972. These birds are considerably smaller than the current broilers, and thus using these birds as a comparison with the modern day broiler would provide an interesting insight into the effect that intensive selection had has on both feeding intake, and the organisation of short-term feeding behaviour. It would also allow us to estimate the effects of past selection programmes on the genetic parameters, such as heritability, of the feeding behaviour traits.

At a genomic level, the association study has indicated areas of the genome linked to feeding behaviour traits. The next stage of this analysis should be to further investigate these regions of the genome, with the aim of identifying genes which are important in the control of feeding behaviour. This would greatly aid in understanding the mechanisms involved in the control of feeding behaviour. Also, genomic analysis allows the possibility of marker-assisted selection, which would greatly improve the accuracy of estimated breeding values used to select the best birds to breed from (Meuwissen and Goddard 1996). As only one line in this study had data available for genomic association, it would be beneficial in the future to compare these results to those in the other lines, to estimate if there are any differences at the genomic level in feeding behaviour in these lines. As an extension of this, it would be interesting to compare these results to those of other poultry species. The turkey genome project is currently underway, and a draft sequence has been published (Reed et al., 2005), and a genetic linkage map has also been published for ducks (Huang et al., 2006), thus SNP analysis for these species will hopefully be possible in the near future.

7.1 Conclusions

In this thesis, the short-term feeding behaviour of three poultry species was analysed, using data obtained from electronic recordings of feed intake from flock-housed individual birds. As existing models to describe STFB (e.g. Yeates et al., 2001) could not be applied to the poultry data, a new model was developed to estimate a biologically-relevant meal criterion when only one or neither population of intervals between visits to feeders is describable (Chapter 2). This model was used to assess whether there had been a change in the hunger and/or satiety mechanisms in four lines fo broilers, which differed in their intensity of selection for growth and feed conversion ratio (Chapter 3). It was thought more likely than the birds which had been more intensively selected for growth may have become more hungry, but no evidence was found for any increase in hunger in any of these lines.

Comparisons between broilers, ducks and turkeys showed that all three poultry species showed a similar structure of STFB, which could be grouped into distinct bouts, after adaptation of current models for estimating meal criteria (Chapter 4). Despite differences in the ages of the birds and the equipment used to record the feeding behaviour, the fitting of a biologically-relevant meal criterion meant that meaningful comparisons could be made between the three species.

At a genetic level, feeding behaviour traits were found to be moderately to highly heritable across all four chicken lines. Some traits, such as meal size and number of

meals per day, had a very high genetic correlation, and this was reflected by the SNP association analysis, which showed that these traits had significant associations with the same regions of the genome. This analysis has highlighted the areas of the genome on which future investigations should concentrate when investigating the genomic control of short-term feeding behaviour and feed intake.

In conclusion, this study has increased understanding of short-term feeding behaviour in broilers and has opened up possibilities for both further academic research on the control of feeding behaviour and feed intake and commercial opportunities to improve feed efficiency .

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Appendix

Appendix

Table 1: Nutritional Specification for Ross 308 Broiler grower diet fed to birds in this study

AMINO ACIDS	Total	Digestible
Lysine %	1.24	1.1
Methionine and cystine %	0.95	0.84
Methionine %	0.45	0.42
Threonine %	0.83	0.73
Valine %	0.96	0.84
iso-Leucine %	0.85	0.74
Arginine %	1.27	1.14
Tryptophan %	0.2	0.18
Crude Protein %	21-23	

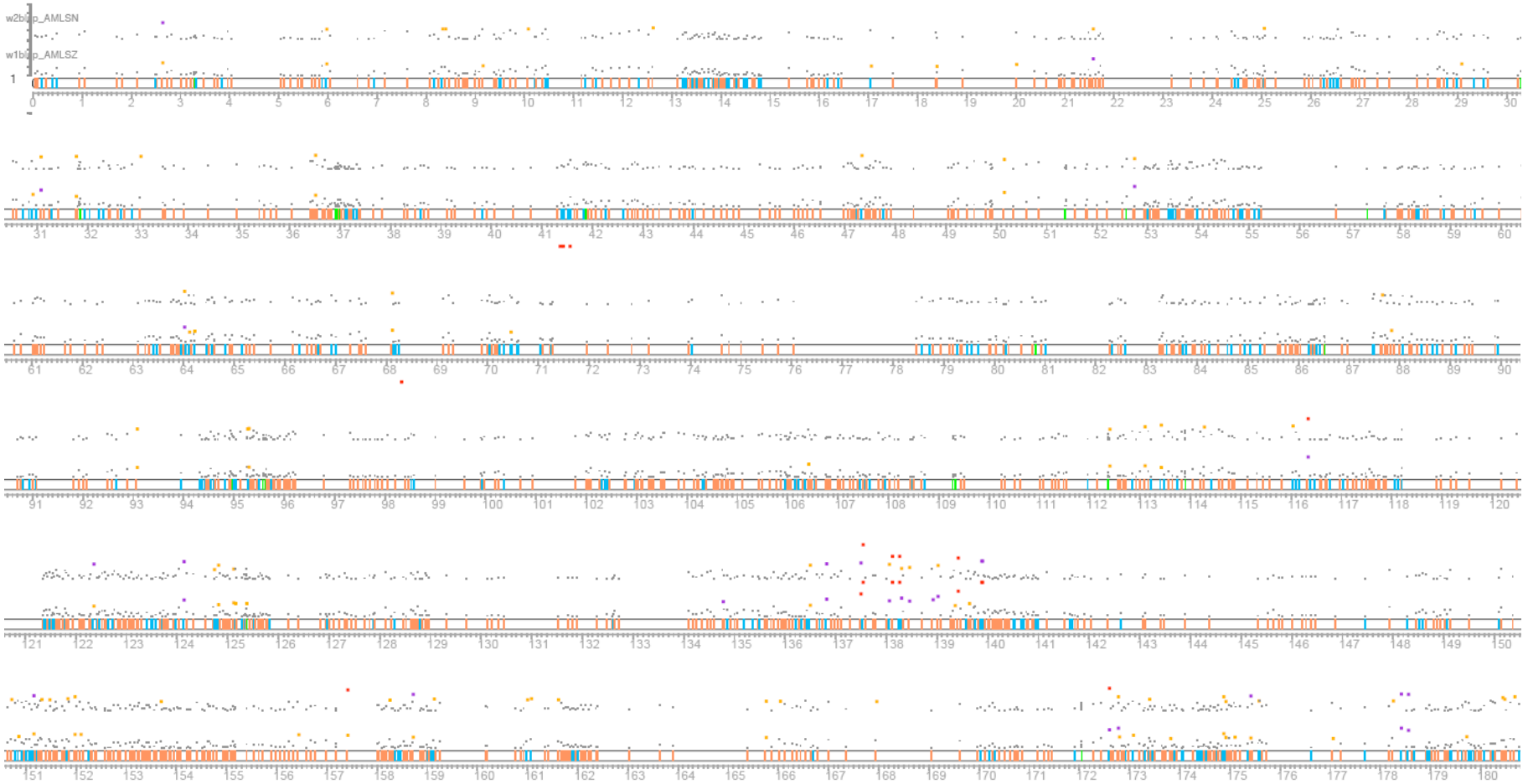
MINERALS	
Calcium %	0.9
Available Phosphorus %	0.45
Magnesium %	0.05-0.50
Sodium %	0.16-0.23
Chloride %	0.16-0.23
Potassium %	0.4-0.9

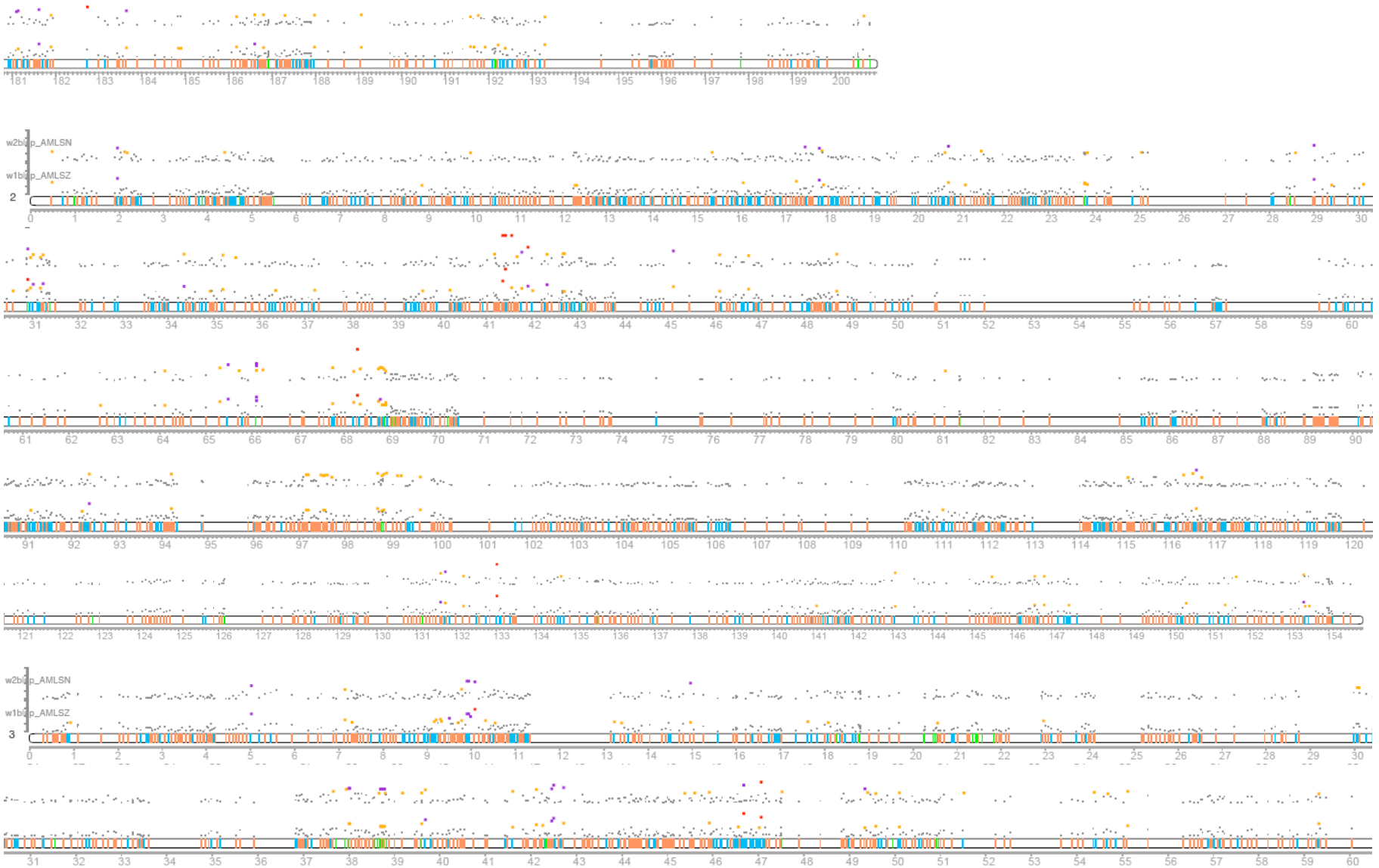
ADDED TRACE MINERALS PER KG	
Copper (mg)	16
Iodine (mg)	1.25
Iron (mg)	40
Manganese (mg)	120
Selenium (mg)	0.3
Zinc (mg)	100

ADDED VITAMINS PER KG	
Vitamin A (iu)	10000
Vitamin D3 (iu)	5000
Vitamin E (mg)	50
Vitamin K (mg)	3
Thiamin (mg)	2
Riboflavin (mg)	6
Nicotinic Acid (mg)	55
Pantothenic Acid (mg)	13
Pyridoxine (mg)	4
Biotin (mg)	0.2
Folic Acid (mg)	1.75
Vitamin B12 (mg)	0.016

Appendix

Figure A1: Genome-wide SNP associations for meal size (MLSZ) and number of meals per day (MLSN). Traits are given on the left-hand side of the diagram for each chromosome and distances were measured in megabases (Mb). Dots on the figure show the level of significance (yellow: p<0.05, purple: p<0.01, red: p<0.001). Bars indicate location of the SNPs (orange: SNP outside known gene, light blue: SNP within known gene, green: SNP within gene previously found to be associated with feed intake).

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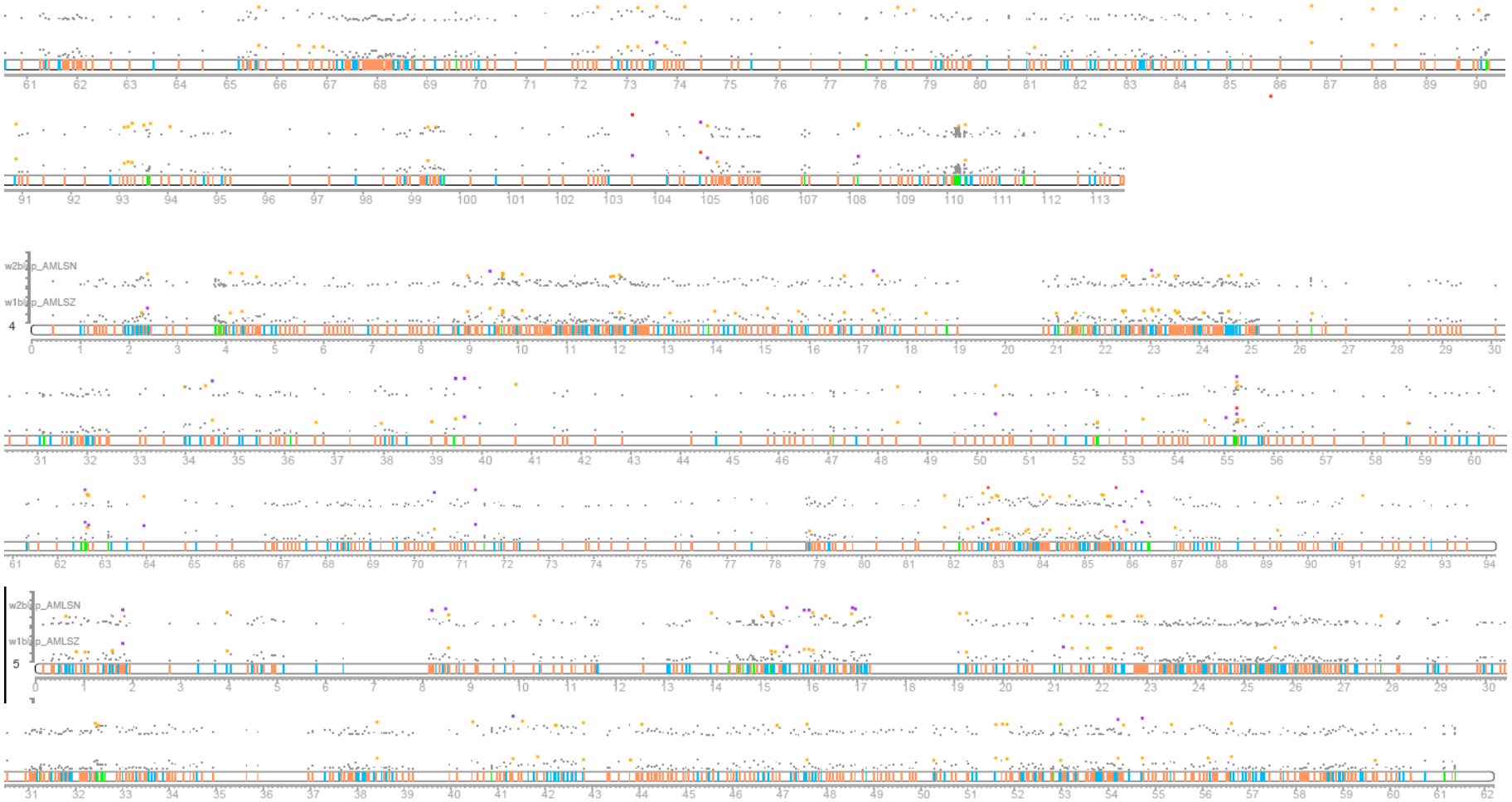
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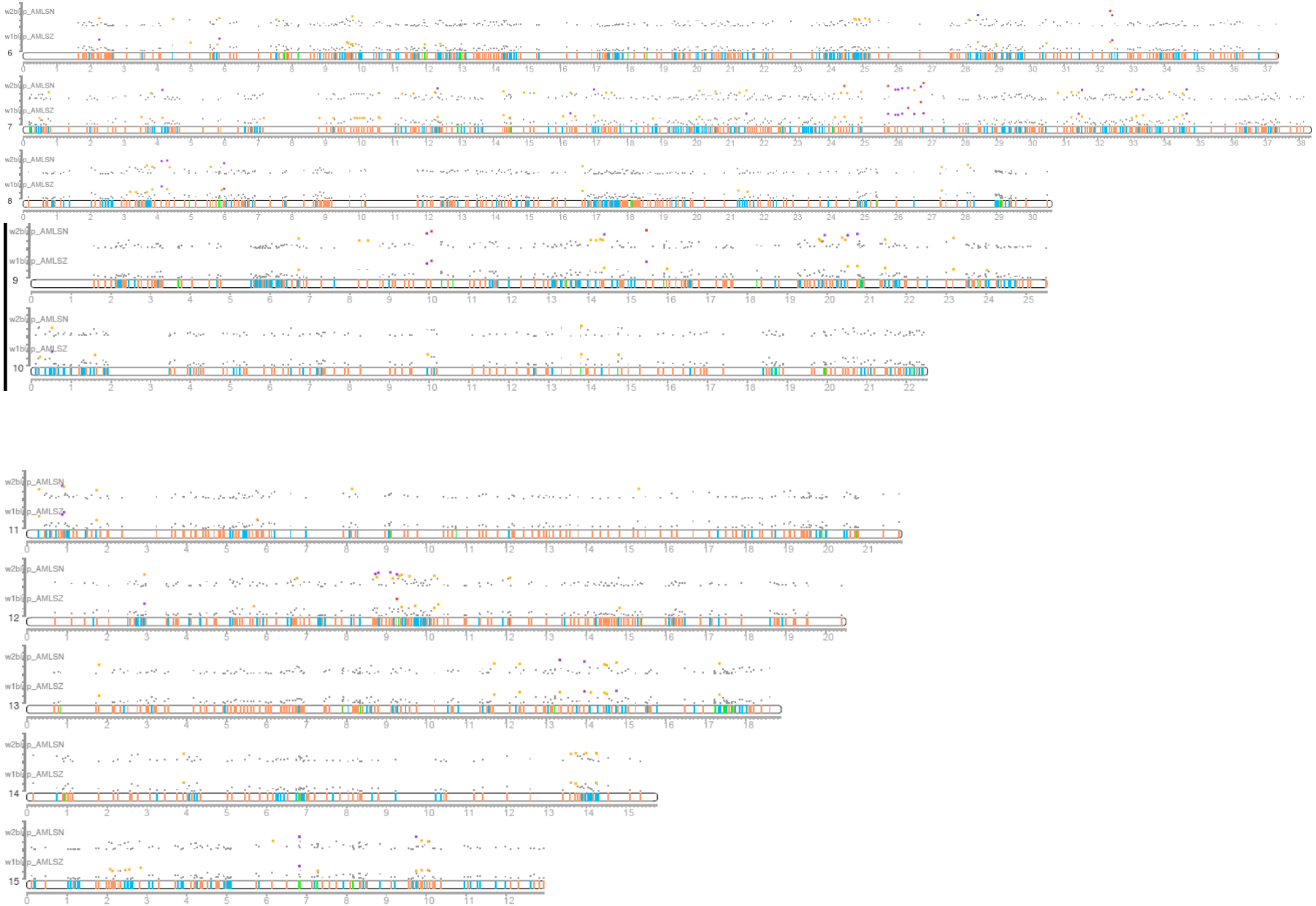
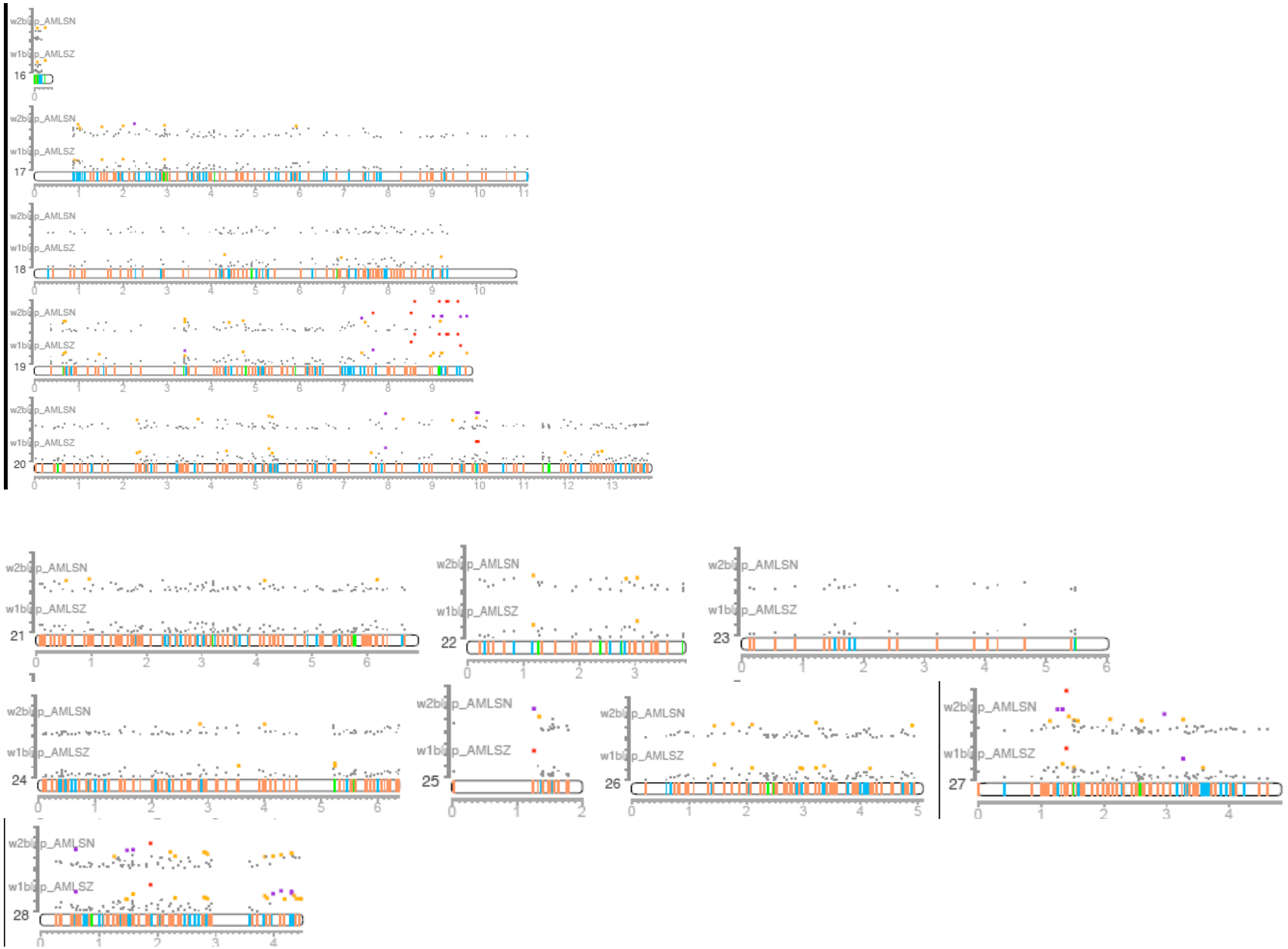
w2b_AMLSN
w1b_AMLSZ
5

Appendix

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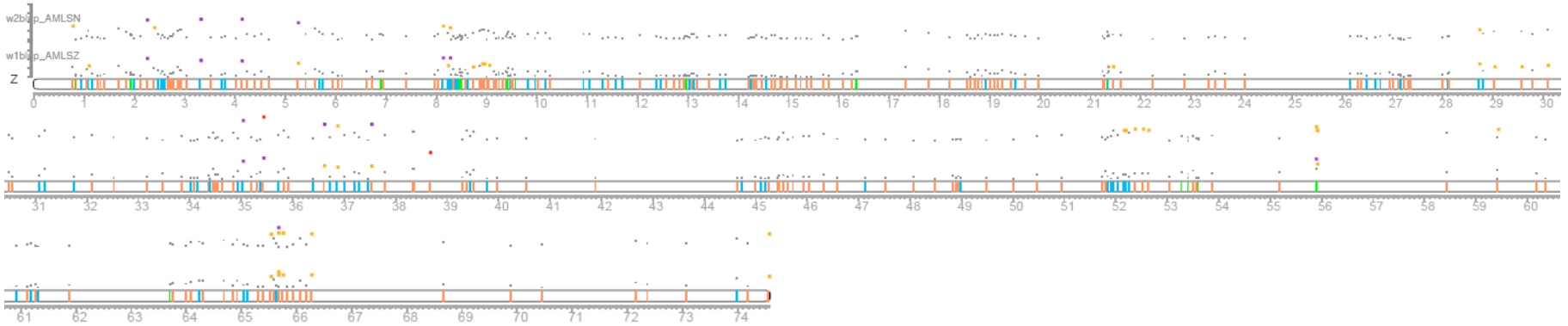
Appendix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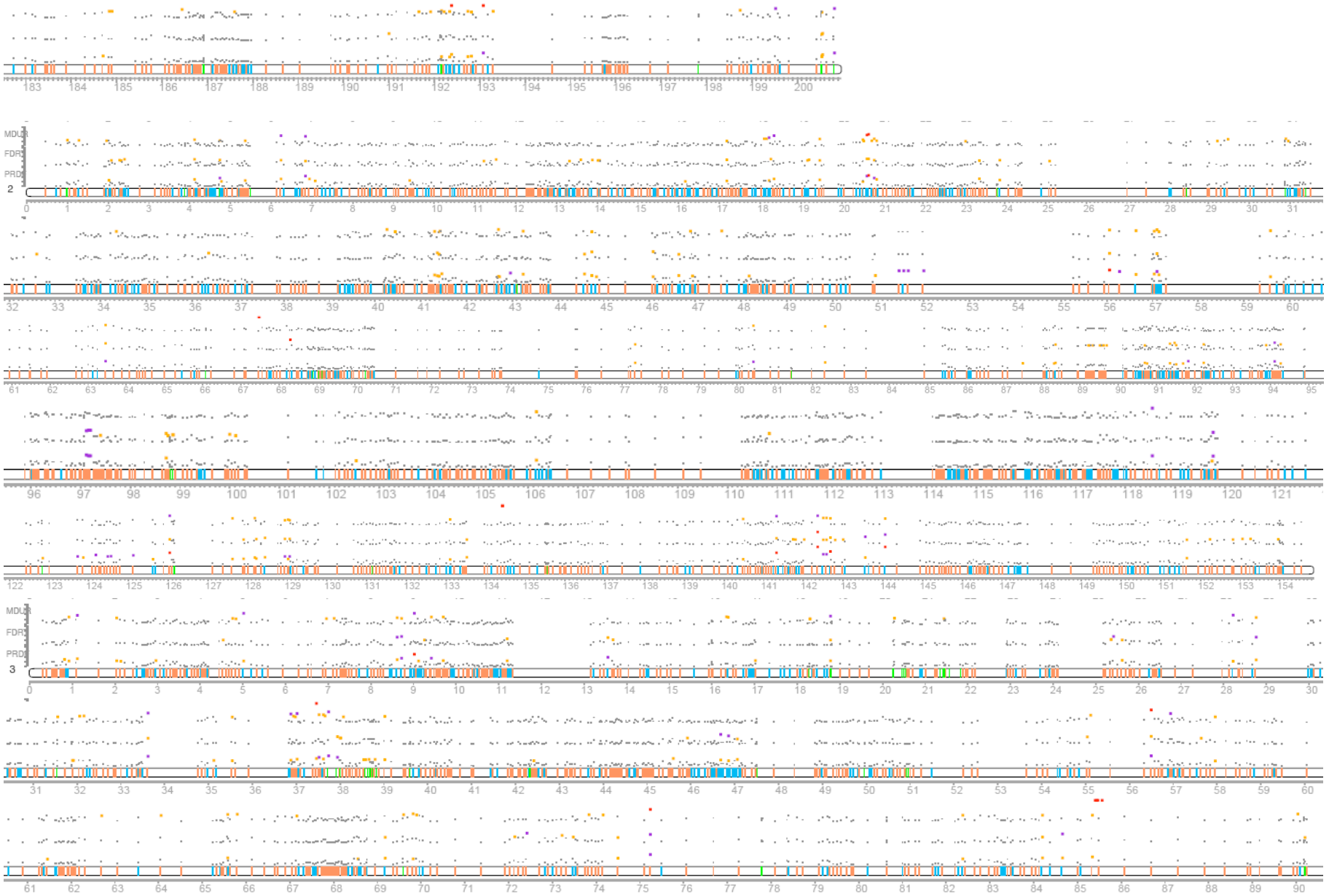
61 62 63 64 65 66 67 68 69 70 71 72 73 74

Figure A2: Genome-wide SNP associations for feeding rate (FDRT), meal duration (MDUR) and time spent feeding per day (PRDF). Dots on the figure show the level of significance (yellow: p<0.05, purple: p<0.01, red: p<0.001). Bars indicate location of the SNPs (orange: SNP outside known gene, light blue: SNP within known gene, green: SNP within gene previously found to be associated with feed intake).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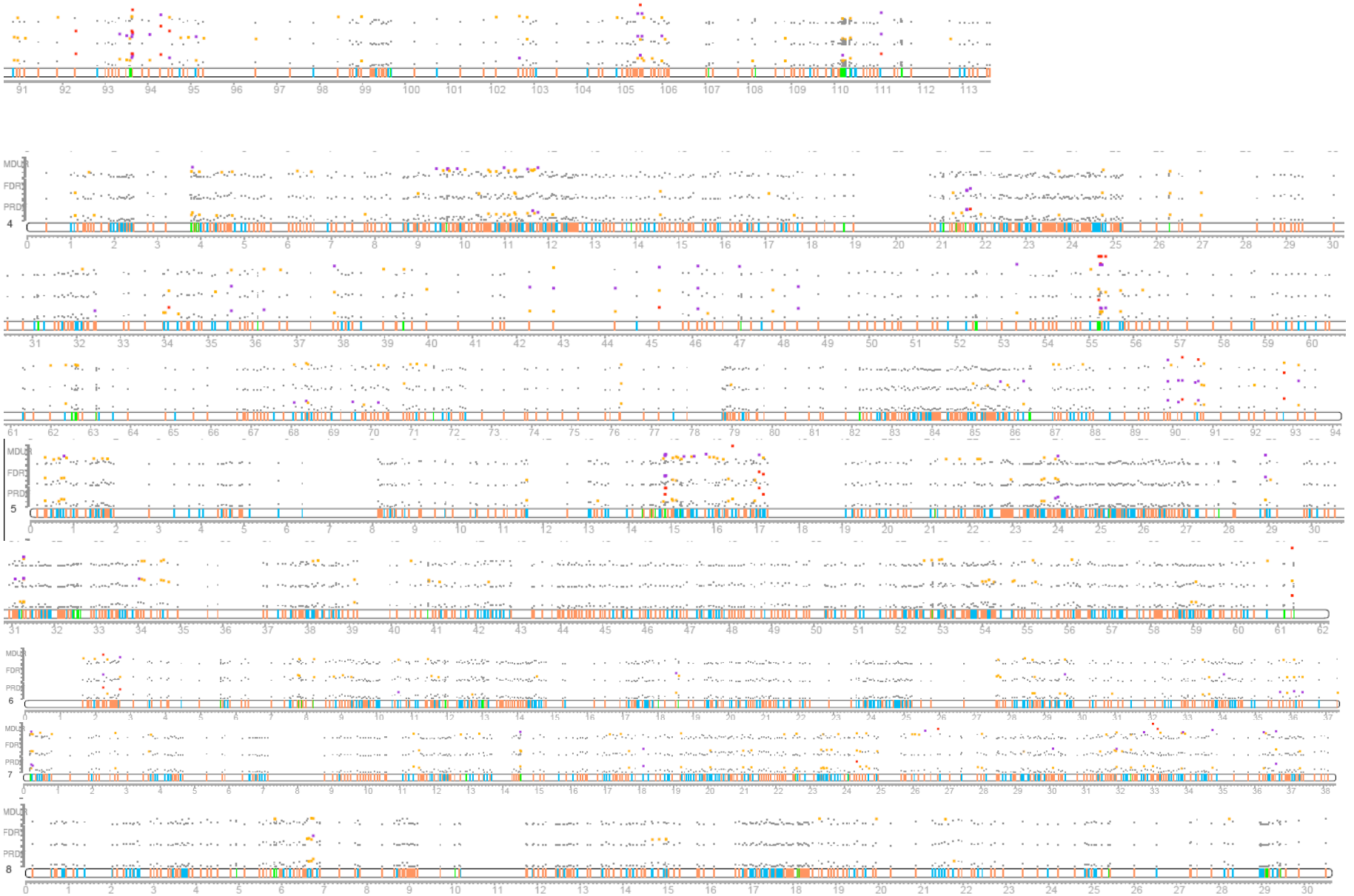
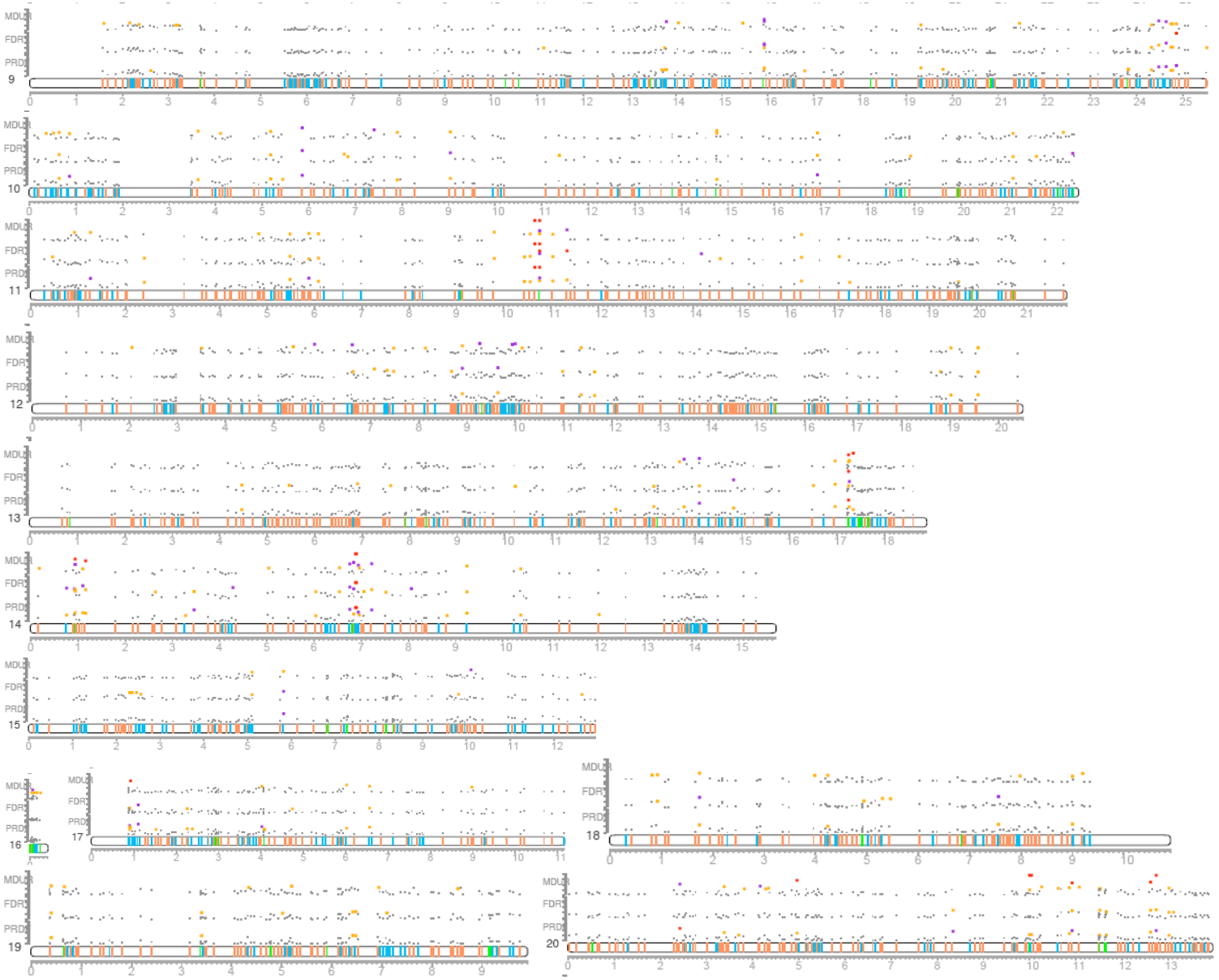
MDU3
FDR1
PRD1
3

Appendix




MDUR
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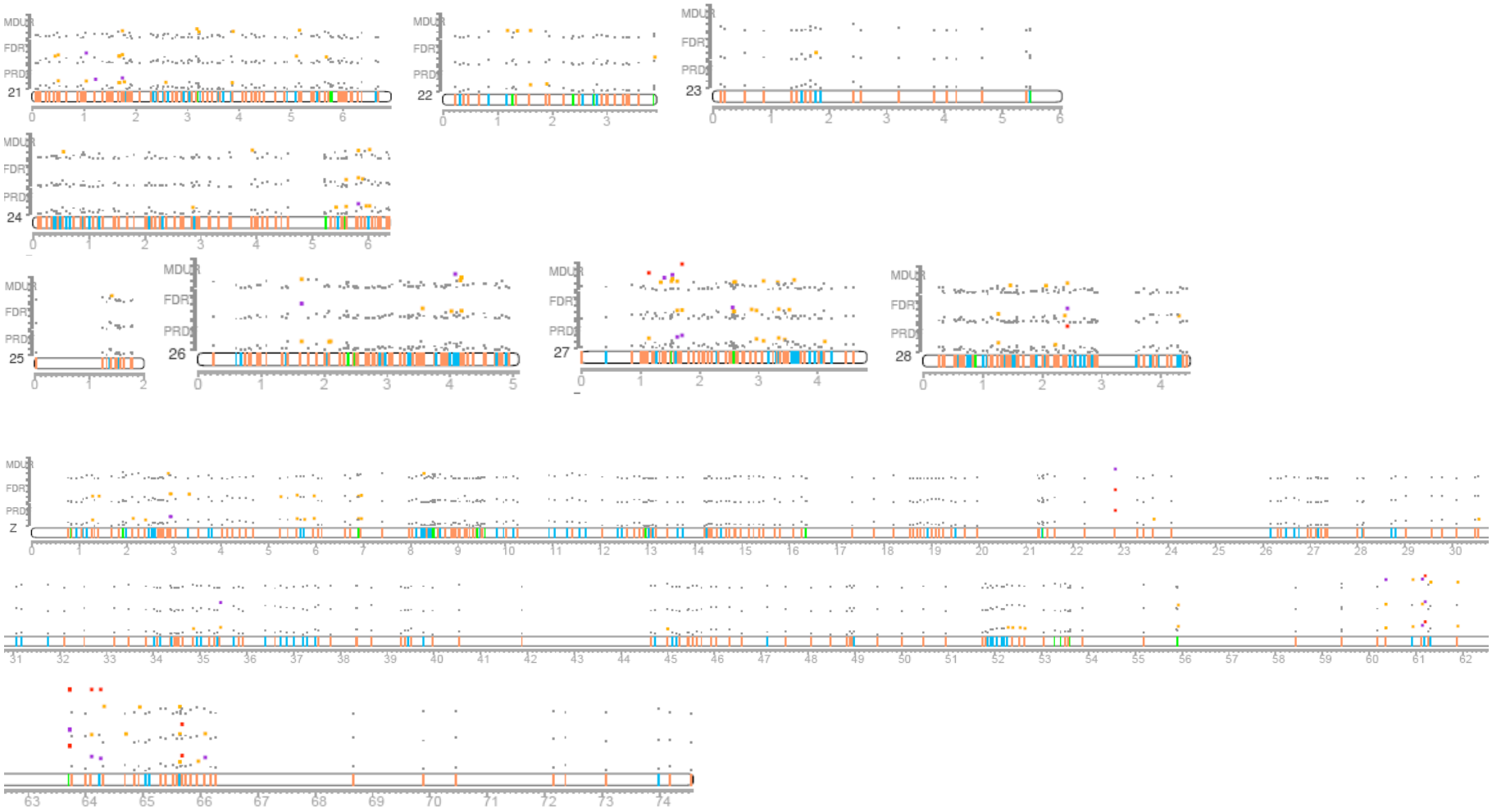
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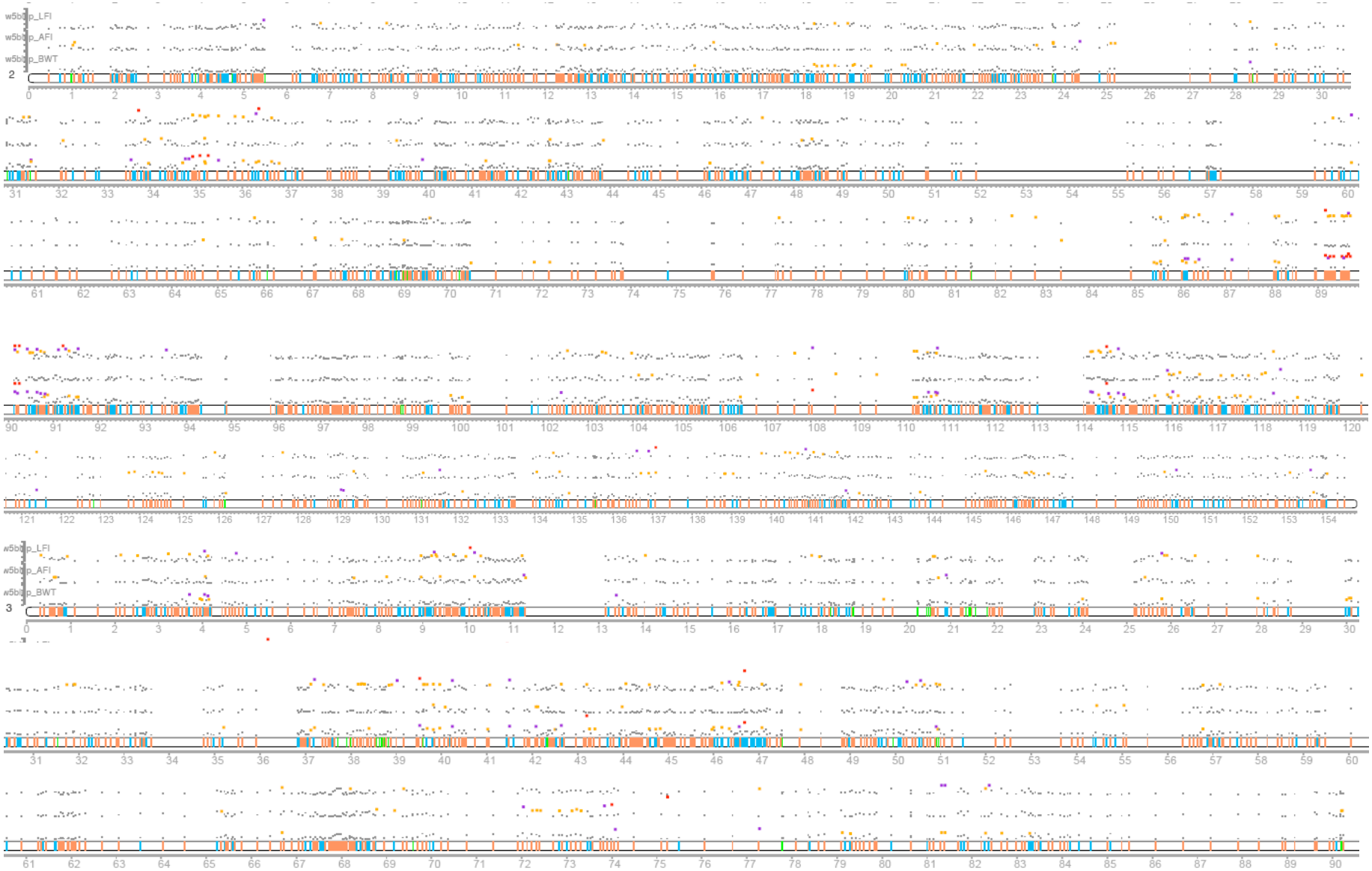
Figure A3: Key areas of the genome with most significant SNP associations with feed intake traits (AFI and LFI) independent of body weight (BWT). Dots on the figure show the level of significance (yellow: p<0.05, purple: p<0.01, red: p<0.001). Bars indicate location of the SNPs (orange: SNP outside known gene, light blue: SNP within known gene, green: SNP within gene previously found to be associated with feed intake).

Appendix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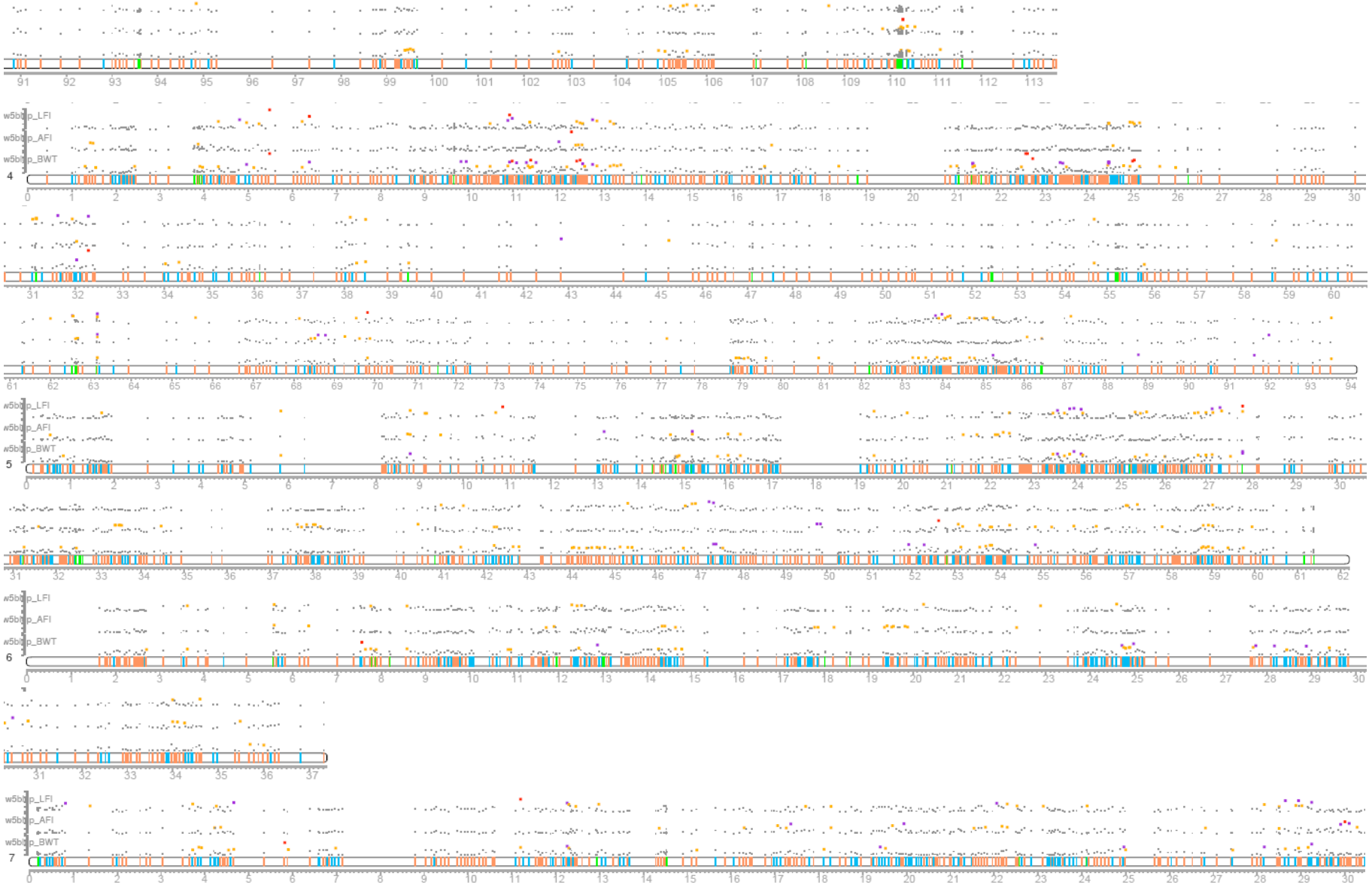
w5bbp_AFI
w5bbp_BWT
2
w5bbp_LFI
w5bbp_AFI
w5bbp_BWT
3


w5bbp_LFI
w5bbp_AFI
w5bbp_BWT
4
5
6
7

Appendix

Appendix

Appendix